Isolating Human Antibody Against Human Hepatocellular Carcinoma by Guided-Selection

Guo-qiang Bao1,t,*
Yu Li2,t
Qing-Jiu Ma1
Xian-Li He1
Jin-Liang Xing2
Xiang-Min Yang2
Zhi-Nan Chen2,*

1Department of General Surgery, Tangdu Hospital; Xi’an, China
2Cell Engineering Research Center; State Key Laboratory of Cancer Biology; Fourth Military Medical University; Xi’an, PR China

†These authors contributed equally to this work.

ABSTRACT

Objective: With the pComb3X-displaying Fab antibody libraries, to achieve the humanization of murine HAB18 against HCC by guided selection.

Methods: With the optimized primers, the human Fd and CL repertoire genes were amplified by RT-PCR from PBMC of HCC patients. The Fd repertoire genes were paired with murine HAB18 CL gene to construct pComb3X-displaying hybrid Fab library. The recombinant HAB18GE was used as antigens to select the target antibodies and got the Fd fragments. Then the human CL genes were paired with the selected human Fds to construct human Fab library. After the panning, the complete human Fab antibodies were got and analyzed.

Results: With the murine HAB18 CL gene as template, the heavy chain Fd shuffling was achieved by panning the hybrid Fab library. Then with the selected Fds as template, the human Fabs were obtained through the light chain shuffling. Two of the resulting human Fabs (HuFab2 and HuFab11), with same Fd and different light chains, bound to HAB18G/CD147 specifically. The competitive ELISA, Western blotting, FCM, fluorescent cell staining and so on demonstrated that the human Fabs resembled its parental murine Fab in that they both perhaps recognized the same epitope. Kd indicated (HuFab2 = 210 nm and HuFab11 = 280 nm) the selected Fabs had available affinity.

Conclusion: Through guided-selection, we got the available human Fab antibodies for the subsequent research. These results suggest that guided selection is a promising strategy in murine mAb humanization.

ABBREVIATIONS

PBMC, peripheral blood mononuclear cells; BSA, bovine serum albumin; CDR, complementary-determining regions; CH1, constant heavy chain domain 1; FR1, framework region; Fd, variable region and constant domain 1 of heavy chain (heavy chain part of Fab); CL, light chain; HRP, horseradish peroxidase; IPTG, isopropyl-β-D-thiogalactosidase; mAb, monoclonal antibody; PCR, polymerase chain reaction; pfu, plaque-forming units; cfu, colony-forming units; HAMA, human anti-mouse antibody response; HAB18, monoclonal antibody against human hepatoma; HAB18GE, extracellular domain of hepatoma associated antigen HAB18G/CD147; DAB, diaminobenzidine; Jev, Japanese encephalitis virus; HHCC, human hepatocellular carcinoma cell line; SDS-PAGE, SDS-polyacrylamide gel electrophoresis; SFDA, State Food and Drug Administration

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common malignant diseases in the worldwide. In China, HCC is one of the most three common cancers relating death.1-3 The death rate of HCC has been rising slowly in the past 20 years. Metastasis of the cancer and the low diagnosis rate of early lesions are the major reasons. The curative surgical operation is the most important therapeutic approach for HCC. But there are still no effective measures for those advanced unresectable tumors as well as metastatic or recurrent ones. HAB18 is a murine IgG1. Antibody against HAB18G/CD147 which expresses highly on the HCC tissue. It does not have cross-reaction with normal liver cells, and only rarely with other malignant tissues. HAB18GE is a mono-Clonal antibody against human hepatoma; HAB18GE, extracellular domain of hepatoma associated antigen HAB18G/CD147; DAB, diaminobenzidine; Jev, Japanese encephalitis virus; HHCC, human hepatocellular carcinoma cell line; SDS-PAGE, SDS-polyacrylamide gel electrophoresis; SFDA, State Food and Drug Administration
antibodies. \textsuperscript{5,6} But complete human mAb will be the best choice for the usage in human. In particular, usage of completely human antibodies, which elicit no or minimal immune response when administered to patients, is yielding an increasing list of FDA-approved protein-based drugs.\textsuperscript{7}

The occurrence of the techniques of guided selection and chain shuffling makes it possible to shift the mouse mAb to human mAb with the same epitope.\textsuperscript{8-11} Here we report our investigation on isolating human anti- HAb18G/ CD147 Fab antibody fragments by subsequently shuffling the murine Fd and CL.

**MATERIAL AND METHODS**

Optimization and identification of PCR primers. We designed a new set of primers to amply human antibody genes based on the different primers used in the relevant studies\textsuperscript{12-15} and the sequences information of FR1 of human germ-line V genes got from the V-BASE (www.mrc-cpe.cam.ac.uk). Briefly, according to the homology of the FR1, the germ-line V genes were divided into different subfamilies. Based on the subfamilies, the primers were designed and optimized with high match rate with the FR1. At the same time, the restriction sites were inserted for the cloning into the vector. All the germ-line V genes were aligned with the primers, the homology and potential amplification efficiency were analyzed. The reverse primers of CH1, CK and CA were according to the reported paper.\textsuperscript{15}

The PBMC used for preparation of total RNA were obtained from peripheral blood of 20 patients with HCC, which were confident with biopsy and physical analysis. PBMC were separated by density gradient centrifugation, and total RNA was separated using an RNA isolation kit (Invitrogen). RNA quality is assessed by agarose gel electrophoresis and spectrophotometry. cDNA was prepared with a first-strand DNA synthesis kit (Promega). PCR amplifications were carried out in 50 µl volumes, containing 5 µl of cDNA reaction product, 0.2 µM of each primer, 200 µM dNTPs, 5 µl 10 × PCR buffer, and 1 U of Taq Plus polymerase (TaqRa). Each variable heavy and light chain back primer was combined with the corresponding constant region reverse primer. The thermal cycle was 95°C for 1 min, 55°C for 1 min, 72°C for 1 min (30 cycles), and followed by 72°C for 10 min (PTC-200, MJ RESEARCH). The amplified DNA fragments were analyzed and purified by electrophoresis on 1% agarose gel.

**Construction and panning of hybrid phage Fd-HAb18cL libraries.** The vector pComb3X was kindly given by Dr. Barbas.\textsuperscript{16} Hybrid Fab libraries containing the murine HAb18 light chain (HAb18cL) and human Fd repertoire were constructed as described with a modification.\textsuperscript{17,18} Briefly, for constructing hybrid Fd-HAb18cL libraries, human Fd PCR fragments were digested with Spe I/Xho I and ligated to linearized pComb3X vector carrying the murine HAb18cL gene. Following ligation, DNA was transformed by electroproporation into E. coli XL1-Blue. After transformation, 3 ml of SOC medium was added and the culture was shaken at 225 rpm for 1 h at 30°C. At this point, samples (1, 0.1 µl) were withdrawn for plating to determine the library size. The remaining bacteria were pelleted on 176-cm\textsuperscript{2} dishes and incubated overnight at 30°C, the colonies were scraped off one plate into 200 ml 2 x YT containing 100 µg/ml kanamycin. The culture was incubated with shaking at 30°C overnight. The supernatant was pelleted by centrifugation (3500 g for 15 min) at 4°C. Phage pellets were resuspended in 200 ml 2 x YT containing 100 µg/ml ampicillin, 20 µg/ml tetracycline and 1% glucose prepared as described with minor modifications.\textsuperscript{19} The culture was shaken at 30°C for 1.5 h. The phagemid vectors were superinfected with helper phage M13KO7 (10\textsuperscript{12} pfu, NEB) for 2 h at 37°C. The plaque was washed twice with water and blocked by completely filling it with 1% BSA in PBS and incubating the plate at 37°C for 1 h. Then 100 µl of the phage library (typically 10\textsuperscript{12} pfu) were added, and the plate was incubated at 37°C for 2 h. Phage were removed and the well was washed with PBS-0.05% Tween five times in the first round, ten times in the 2nd round and 15—20 times after the 3rd round.\textsuperscript{21} The plate was washed once more with distilled water and adherent phages were eluted by the addition of 100 µl of elution buffer (0.1 M HCl, adjusted to pH 2.2 with solid glycine and containing 0.1% BSA) and incubation at room temperature for 10 min. The elution buffer was pipetted up and down several times, removed, and neutralized with 6 µl of 2 M Tris base. Eluted phage were used to infect 2 ml of fresh E. coli XL1-Blue cells (OD\textsubscript{400 nm} = 0.8–1.0) for 15 min at room temperature. The bacteria were plated on 176-cm\textsuperscript{2} dishes and incubated overnight at 30°C for next round panning to obtain the human Fds.

**Construction and panning of human phage Fab libraries.** The selected human Fd genes were digested with Spe I/Xho I and ligated to linearized pComb3X vectors. Then the new vectors pComb3X/hFd containing the selected human Fd genes were used to construct human phage Fab library. For construction of the human Fab libraries, human C\textsubscript{\gamma} PCR fragments were digested with Sac I/Xba I and ligated to linearized pComb3X/hFd vectors.

The human Fab library was constructed and panned as 1.2 (Fig. 1).

**Preparation soluble PIII-fusion Fab fragments.** After panning, single colonies were grown separately in 2 ml 2 x YT medium containing 100 µg/ml ampicillin, 20 µg/ml tetracycline and 1% glucose at 30°C. For soluble Fab preparation, colonies were grown in 20 ml of 2 x YT containing 100 µg/ml carbenicillin, 20 µg/ml tetracycline and 1% glucose at 30°C until OD\textsubscript{600} of 0.6 was achieved. Cells were pelleted by centrifugation at 4000 rpm for 10 min at 4°C and resuspended in 10 ml 2 x YT containing 100 µg/ml carbenicillin, 20 µg/ml tetracycline. IPTG (1 mM) was added and the culture was incubated overnight at 30°C. Cells were pelleted by centrifugation at 4000 rpm for 10 min at 4°C and washed one time with 2 ml PBS. Cells were pelleted by centrifugation at 4000 rpm and resuspended in 1 ml PBS. The cells were given 3–4 times freeze-thawing. The lysate was pelleted by...
The HHCC and NIH3T3 were used as the control.

Forward primers used for construction of the library

| Primer Name | Variable region amplification primers | Length (bp) | Matches |
|-------------|---------------------------------------|-------------|---------|
| IgHV1:      | 5’(-GC)AG CTC GAG CAG CAG TCT GG-3’   | 23          | V₁,1,4  |
| IgHV2:      | 5’(-GC)GTC CTC GAG CAG TCT GG-3’     | 23          | V₁,2    |
| IgHV3:      | 5’(-GC)AG CTC GAG CAG TCT GG-3’     | 23          | V₁,4    |
| IgHV4:      | 5’(-GC)AG CTC GAG CAG TCT GG-3’     | 23          | V₅,5,7  |
| IgHV5:      | 5’(-GC)AG CTC GAG CAG TCT GG-3’     | 23          | V₅,6    |
| IgKV1:      | 5’(-GC)AG CTC GAG CAG TCT GG-3’     | 23          | V₅,14   |
| IgKV2:      | 5’(-GC)AG CTC GAG CAG TCT GG-3’     | 23          | V₅,2    |
| IgKV3:      | 5’(-GC)AG CTC GAG CAG TCT GG-3’     | 23          | V₅,3    |
| IgKV4:      | 5’(-GC)AG CTC GAG CAG TCT GG-3’     | 23          | V₅,3,6  |
| IgKV5:      | 5’(-GC)AG CTC GAG CAG TCT GG-3’     | 23          | V₅,1    |
| IGLV1:      | 5’(-GC)AG CTC GAG CAG TCT GG-3’     | 23          | V₅,1    |
| IGLV2:      | 5’(-GC)AG CTC GAG CAG TCT GG-3’     | 23          | V₅,2    |
| IGLV3:      | 5’(-GC)AG CTC GAG CAG TCT GG-3’     | 23          | V₅,2    |
| IGLV4:      | 5’(-GC)AG CTC GAG CAG TCT GG-3’     | 23          | V₃,3    |
| IGLV5:      | 5’(-GC)AG CTC GAG CAG TCT GG-3’     | 23          | V₃,3    |
| IGLV6:      | 5’(-GC)AG CTC GAG CAG TCT GG-3’     | 23          | V₄,4    |
| IGLV7:      | 5’(-GC)AG CTC GAG CAG TCT GG-3’     | 23          | V₄,5    |
| IGLV8:      | 5’(-GC)AG CTC GAG CAG TCT GG-3’     | 23          | V₅,5    |
| IGLV9:      | 5’(-GC)AG CTC GAG CAG TCT GG-3’     | 23          | V₆,6    |
| IGLV10:     | 5’(-GC)AG CTC GAG CAG TCT GG-3’     | 23          | V₇–10   |

Centrifugation at 10000 g for 5 min. The soluble Fabs in the supernatant were used for ELISA analysis and cell staining.

Soluble PIII-fusion Fabs ELISA for positive clone identification. The binding specificity of the selected clones was determined by ELISA using the target antigen. Ten µg/ml antigen (HAb18GE, BSA, GST) in 100 µl of 0.05 M bicarbonate buffer (pH 9.6) was coated onto ELISA plates by overnight incubation at 4°C. Soluble PIII-fused Fabs mixed with 1% BSA were incubated at 37°C for 2 hr and added into plate wells to incubate for 1 hr at 37°C. Then 50 µl of 1/200 diluted HRP/goat anti-human IgG conjugate (Pharmacia) in blocking buffer was added followed by color development with TMB substrates.

The supernatant was mixed with diluted parental antibody HAb18 (0.001 ng/ml, 0.01 ng/ml, 0.1 ng/ml, 1 ng/ml, 10 ng/ml, 100 ng/ml) for competitive ELISA. The mAbs against Jev and AFP are used as the control.

Fluorescent cell staining with soluble PIII-fusion Fabs. HHCC cells that express the antigen HAb18g/CD147 highly and murine fibroblast cell (NIH3T3) that does not express the antigen HAb18g/CD147 were plated at a density of 5 x 10³/ml on glass slides in dishes and cultured overnight. Cells on slides were fixed with cold acetone for five minutes, and blocked with 5% non-immune goat serum for 30 min at 37°C. After the blocked and washed, soluble Fabs in the supernatant (50 µl) were incubated with fixed cells for 1 hr at room temperature. For HHCC, FITC conjugated anti-human IgG antibodies (Southern Bio) were subsequently incubated with the slides at 37°C for 45 min. For NIH3T3, FITC conjugated antibodies were diluted in Evan's blue buffer before incubating with the slides. Then the slides were observed under fluorescence microscope.

Western Blotting. After blocking, the membranes (Millipore, Watford, UK) for Western blotting were cut into strips and each strip was incubated with fixed cells for 1 hr at room temperature. For HHCC, FITC conjugated coat F(ab), anti-human IgG antibodies (Southern Bio) were subsequently incubated with the slides at 37°C for 1 hr. The strips were washed followed by development with DAB as HRP substrate.
paraffin-embedded tissue sections. Sections were incubated with the selected human Fabs as primary antibodies. HRP-conjugated anti-human Fab antibody (Sigma) was added sequentially followed by development with DAB as HRP substrate.

**Nucleotide sequence determination.** The selected Fd and C\textsubscript{\gamma} genes were inserted into pBluescripts KS (Promega) for sequence analysis. Products of the sequencing reaction were analyzed on an automated sequencer (TM377, ABI). Oligonucleotides used were T3 primer: 5'-AAT TAA CC TCA CTA AAG GG-3' and T7 primer: 5'-GTA ATA CGA CTC ACT CTA GGG C-3'. DNA sequences were aligned and analyzed with BLAST (www.ncbi.nih.nlm.gov).

**Purification of Fabs and determination of affinity index.** For soluble Fab preparation, phagemid DNA from positive clones was isolated and digested with Nhe I and the resulting 4.3 kb DNA fragment lacking the gene III portion was self-ligated. Following transformation of E. coli/TG-1, colonies were grown in 20 ml of 2 x YT containing ampicillin (100 μg/ml) at 37°C until OD\textsubscript{600} of 0.2 was achieved. IPTG (1 mM) was added and the culture was incubated overnight at 30°C. Cells were pelleted by centrifugation at 20000 g for 5 min at 4°C. The soluble Fab in the supernatant were purified by chromatography column SP-40HR.10 Surface plasmon resonance (SPR) was used to measure the binding kinetics of Fabs to HAb18GE on a BIACore instrument.22,23 Briefly, the purified HAb18GE was covalently immobilized to a CM5 sensor chip via amine group using the amine coupling kit (BIACore) in 10 mM sodium acetate buffer, pH 6.0. Experiments were run at a flow rate of 5 μl/min in HEPES-buffered saline containing 10 mM HEPES (pH 7.4). The surface was regenerated with 100 mM hydrochloric acid and 0.2 M Tris buffer. Binding kinetic parameters were measured with antibodies at different molar concentrations and evaluated with BIACORE-EVALUATION software (BIACore).

**RESULTS**

**Optimization and identification of primers.** In order to achieve access to as many different human heavy and light chain V-region gene segments as possible, a new set of oligonucleotide primers was developed (Table 1), the design of which was based on the most recent sequence information provided by the V-BASE. According to the human germ-line V-genes in the V-BASE, we designed a set of specific 5, primers, including five primers for the Fd genes, five primers for the kappa chain and ten primers for lambda chain. All the germ-line V-genes are aligned with the 5' primers. Sequence homologous analysis showed that the forward VH primers, VK and VL primers covered 80.8% and 69.2% and 82.8% of V germ-line genes respectively with ≤ 3 mismatch bases. And compared with the last eight bases, the VH, VK and VL primers covered 88.2%, 100% and 74.4% of V germ-line genes at 100% homology respectively.

Then with the primers, we amplified the genes of Fd and light chains.24 PCR showed that all the primer-pairs could be used to amplify the target antibody genes (Fig. 2). The genes of ten clones were sequenced to assess the primers specificity. Genes sequencing confirmed the most interested genes could be amplified with good diversity. The set of optimized family-specific primers can amplify the human genes of the Fd and light chains with a high efficiency. These will be helpful for the construction of human Fab antibody libraries.

**The construction of phage antibody and guided selection.** In the study, according the richness of the VH genes, the corresponding VH forward primers were divided into three groups(1\textsuperscript{st} group: IGHV1/3; 2\textsuperscript{nd} group: IGHV4; 3\textsuperscript{rd} group: IGHV2, V5/7, V6). So the PCR products of VH genes were divided into 3 groups. Depending on the group, we constructed three hybrid Fab library containing murine light chain, then mixed them to get the target Fab library for the panning. In the study, we constructed the hybrid Fab library containing 2 x 10\textsuperscript{9} pfu. Through the PCR analysis, the insertion rate of target fragment was 90%. At the start stage, the GST fused HAb18GE were used for the panning.25 But the fusion protein could be react with the blank plasmid. So we turned to use the purified HAb18GE to screen the library. Through six rounds panning, we obtained seven specific clones with higher positive signal.

The different Fd was mated with different C\textsubscript{\gamma} . The conformation would affect the affinity of the assembled Fab. To remove the disturbance of the affinity descending because of the mismatch VH and VL pair, the selected seven Fd genes were inserted the intact vector for the construction of human Fab library. In the study, we constructed a human Fab library containing 0.8 x 10\textsuperscript{7} pfu. Through the analysis of endonucleases digestion, the insertion rate of target fragment was >90%. Through four rounds panning with

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**Figure 3.** Competition of plI fused Fabs in supernatant with murine HAb18 by ELISA. (A) HuFab2; (B) HuFab11; Maximal absorbance (OD490) of a sample with plI fused Fab supernatant in the absence of a competitor was set to standard value. All samples were analyzed in triplicate. The mean values and standard deviations (SD) of representative experiments are shown.

**Figure 4.** The plI fusion human Fab in the incubation supernatant was used for the immunofluorescence staining as the first antibody. For HHCC cells expressing HAb18G/CD147 highly, the membrane could be stained with the primary antibody added (A1, HuFab2; A2, HuFab11; A3, no primary antibody treated; ×400). For NIH3T3 cells, there are no significant staining with or without the primary antibody (B1, Fab2; B2, Fab11; B3, no primary antibody treated; ×400).
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purified HAb18GE, we obtained 2 clones (HuFab2 and HuFab11) with higher specific signal.

Determining the affinity of selected Fab clones by competitive ELISA. Competitive ELISA was performed to determine if the HuFab2 and HuFab11 recognized the similar epitope of HAb18G/CD147 as the murine parental monoclonal antibody HAb18. Binding of HuFab2 and HuFab11 to immobilized HAb18GE was inhibited in a dose dependent manner by increasing concentrations of the murine HAb18 (Fig. 3). The binding of the selected Fabs could not be blocked by anti-Jev mAb even at a very high concentration (10 µg/ml). These results demonstrated that the guided selected Fab perhaps had similar specificity with HAb18.26

Biological activity of the selected Fabs. As shown in Figure 4, HHCC and NIH3T3 cells were stained by the selected antibodies HuFab2 and HuFab11 as the primary antibodies. For HHCC, fluorescent cell staining showed that the positive signal was located mainly on the cell membranes and no detectable signal in negative control without addition of the primary antibody (Fig. 4A1, A2 and A3). For NIH3T3, only red signal could be detected with the Evan’s blue staining but no positive fluorescent staining be seen (Fig. 4B1, B2 and B3).

Flow cytometry indicated HuFab2 and HuFab11 binding significantly to the cell membrane of HHCC (Fig. 5), but nearly not bind to membrane of NIH3T3 comparing with negative control that added by only the FITC conjugated second antibody.

In the present study, the HHCC and NIH3T3 lysate and purified HAb18GE were used for Western blot analysis(Fig. 6). The parent murine HAb18, Fab2 and Fab11 were used as the primary antibodies respectively. Each antibody could bind to band of 58 kDa on the membrane transferred HHCC lysate but not bind to membrane transferred NIH3T3 lysate. For purified HAb18GE, bands adjacent to 20 kDa could be seen because the protein was expressed in E. coli without the glycosylation. Then the immunohistochemical staining was performed using HuFab2 and HuFab11 as the primary antibody. The results of immunohistochemical staining are presented in Figure 7. HCC tissues were stained when HuFab2 and HuFab11 were used as the primary antibody, but the normal liver tissues were negative.

DNA sequencing and analysis. Two distinct clones were identified whose Fab fragments used the same V_H region paired with two different V_L regions (Table 2). Fd belongs to IgG2 subclass. The sequencing analysis showed that the heavy variable region belongs to human VH3 family. The C_L2 and C_L11 variable region belong to V_K3 family.

Affinity of selected antibodies. Binding kinetic rates ($K_{on}$ and $K_{off}$) and affinities of HuFab2 and HuFab11 for immobilized HAb18GE were measured by surface plasmon resonance. The results showed that the selected human Fabs have little slower association and little quicker dissociation rate constants, $K_{on}$ and $K_{off}$ compared with the parent murine
antibody. The $K_D$ of the clone HuFab2 was 210 nm, which was about three times of the value of the parent Fab. The $K_D$ of the clone HuFab11 was 280 nm, and about four times of that of the parent Fab.

**DISCUSSION**

Targeting diagnosis and therapy of HCC with anti-HCC mAb have been studied extensively, giving a hopeful prospect to HCC treatment. Targeting therapy is a common means of tumor immunotherapy, which is called “biological missile”. Since the discovery of monoclonal antibody in 1975, mAbs have held great promise for the treatment of human disease such as cancer, viral infection, and autoimmune disorders. The most important limitations in murine mAb have been the immune response against murine immunoglobulins and insufficient activation of human effector function. These problems have been overcome using genetic engineering techniques to produce chimeric mouse-human, CDR-grafted, and fully human antibodies.

From 1994, chain shuffling and guided selection are used for humanization of mouse mAb extensively. One of the advantages of the strategy of guided selection compared to direct phage display selection from antibody libraries for generation of human antibody is that it is more feasible to isolate antigen binders from a large antibody gene library. In the study, we made optimization in the procedure of the library construction: (1) We used the PBMC of HCC patient to amplify the Fab genes, so to increase the richness of the interested antibody genes; (2) According the richness of $V_H$ genes, we constructed three hybrid Fab library to decrease the bias of rare clones in the amplification and panning.

In present study, the subsequent chain shuffling was used for the humanization of HAb18. Theoretically, the $10^7$ light chains and Fd chains could form $10^{14}$ possible Fab pairing, a figure far exceeding the possible size of a combinatorial library constructed by electroporation. On the other hand, guided selection, by sequential shuffling each chain of heavy chain and light chain of an antibody, could make full use of each single chain repertoire of up to $10^7$. Fd play the key role in the affinity of Fab, so our humanization strategy of guided selection involves two steps. First, the light chain of HAb18 antibody was, as the template, paired with the human Fd repertoire and selected by immobilized human HAb18GE. pComb3X vector containing murine light chain of HAb18 was used to construct phage display libraries with a human Fd repertoire, which derived from the peripheral blood of 20 HCC patients. Thus the human Fd chain genes were obtained through several rounds of panning. Then, the Fd genes were paired with the human $C_H$ repertoire and selected as above to acquire human Fd genes.

The construction of a large human phage antibody library with good diversity is based on the amplification of all the antibody genes. The first step to select the targeted antibody with the library is to try to amplify all the antibody genes. So the primer design plays an important role in the work. We made an optimization for the primers. Through the compare with other reported primers, and the analysis of efficiency and diversity, the set of primers can be used for the amplification of human Fab genes with good efficiency.

The usage of the GST fusion protein for the panning of antibody library can cause cross reaction. And it can cause the difficulty to identification. In the study, the GST-fusion HAb18GE was used for the panning of the hybrid Fab library to cause disturbance of GST specific positive clones. So in subsequence study, purified no fusion HAb18GE was used for the panning.

The direct ELISA of phage-displaying Fab is used for the identification of the positive clones generally. In the ELISA positive clones, some clones had no the Fd genes insertion with PCR or restriction digestion(the result not showed). Even the plasmid was blocked with extensive GST or BSA, but the phenomena also existed. The cross reaction interfered the panning efficiency. Because of the cross reaction and the complicated rescue procedure, pIII-fusion scFv can be used for the screening of the antibody library. Soluble antibody fragments from the selected clones were usually produced by induction with IPTG in the absence of helper phage. In the present study, the supernatant of lysate was used for the identification of the positive clones.

Our data indicate that human anti-HAb18G antibody can be obtained by guided selection with the murine antibody fragment as
template. Because the selected HuFad2 and HuFad211 are entirely of human origin, it is expected to be much less immunogenic and therefore useful for sequential administration as a therapeutic reagent.

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