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Construction of single-chain variable fragment antibodies against MCF-7 breast cancer cells

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ABSTRACT. A phage display library of single chain variable fragment (scFv) against MCF-7 breast cancer cells was constructed from C3A8 hybridoma cells. RNA from the C3A8 was isolated, cDNA was constructed, and variable heavy and light immunoglobulin chain gene region were amplified using PCR. The variable heavy and light chain gene regions were combined with flexible linker, linked to a pCANTAB 5E phagemid vector and electrophoresed into supE strain of *Escherichia coli* TG1 cells. Forty-eight clones demonstrated positive binding activity to MCF-7 breast cancer cell membrane fragments and the strongest of 48 clones was selected for analysis. The anti-MCF-7 library evaluated by *Sfi*I and *Not*I digests demonstrated that anti-MCF-7 scFv

antibodies possess individual patterns that should be able to recognize distinct human breast cancer cells. The C3A8 scFv, with an apparent molecular weight of 32 kDa, showed high homology (99%) with single chain antibody against rice stripe virus protein P20. In summary, the anti MCF-7 scFv antibody can be used for pretargeting breast cancer for clinical diagnosis of patients; it also has potential for therapeutic applications.

Key words: Recombinant antibody; scFv; C3A8; MCF-7

INTRODUCTION

The advances in antibody cloning technology have greatly facilitated the genetic manipulation of antibody fragments and permitted the development of a large variety of engineered antibody molecules for research, diagnosis and therapy (Boss et al., 1984; Kontermann and Muller, 1999). It is possible for the cloned antibodies to have improved the affinity and specificity of antigen binding by mimicking somatic hypermutation during an immune response (Gram et al., 1992). The generation of single-chain variable fragments (scFv) has become an established technique used to produce soluble antibody in bacterial systems. scFv are constructed mainly from hybridomas (Huston et al., 1988; Chaudhary et al., 1990), spleen cells (Chowdhury et al., 1998; Finlay et al., 2006), and also human B lymphocytes (Shadidi et al., 2001; Zhang et al., 2006). An antibody in scFv format consists of variable regions of heavy (V_H) and light (V_L) chains that are joined together by a flexible peptide linker. It can be easily expressed in functional form in *Escherichia coli*, allowing protein engineering to improve the properties of scFv, such as increased of affinity and alteration of specificity (Griffiths and Duncan, 1998). Apart from bacteria, scFv have been successfully isolated and displayed as fragments in mammalian cells, yeast (Ho et al., 2006), plants (Huston et al., 1996) and insect cells (Choo et al., 2002).

In cancer treatment, radioimmunotherapy (RIT) has shown efficacy in the treatment of advanced metastatic human breast cancer, but the advantageous characteristics of tumor specificity attributed to the use of intact monoclonal antibodies is often diminished due to the increased radiation dose delivered to normal tissues during the extended time that the large antibody molecules remains in circulation. To improve RIT, various versions of genetically engineered multivalent antibodies, including diabodies and minibodies have been investigated (Hu et al., 1996; Adams et al., 1998). scFv have demonstrated to be a particularly useful tool for clinical and medical applications, since they are only half the size of fragment antibodies (Fabs) and thus have lower retention times in non-target tissue, more rapid blood clearance and better tumor penetration (Huston et al., 1996; Marasco et al., 1998). Phage displayed technology is a system that makes it possible to generate scFvs with desired binding affinity and specificities (Garet et al., 2010; Pande et al., 2010). In the present study, we demonstrated the construction of anti-MCF-7 scFv antibodies specific to human breast cancer cells by phage display technology.

MATERIAL AND METHODS

Cell culture

The C3A8 hybridoma cell line was prepared by immunization of a mouse with MCF-7

breast cancer cells and hybridization of the B cells from the spleen of the immunized mouse with the murine myeloma cell line Sp2/0-Ag14 (Ali et al., 1996). The hybridoma cell line producing Mab (isotype IgM, κ) was used as the source for the preparation of scFv while MCF-7 cells were used for the preparation of antigens. Both hybridoma and MCF-7 cells were retrieved from storage in liquid nitrogen and maintained in 10 mL RPMI-1640 medium, supplemented with 10% heat- inactivated fetal bovine serum, 0.2% sodium bicarbonate, 100 U/mL of penicillin and 100 U/mL streptomycin at 37°C in atmosphere of 5% CO_2.

Amplification and cloning of the immunoglobulin V_H and V_L chains

Messenger RNA was isolated from actively dividing hybridoma cells using the Quick-Prep mRNA Purification kit (Pharmacia, Sweden) according to the manufacturer instructions. The purified mRNA was used for first-strand cDNA synthesis and the resulting V_H and V_L chain cDNAs were amplified using a recombinant phage antibody system kit (RPAS; Pharmacia, Sweden). Amplification using 5000 U/mL AmpliTaq DNA polymerase (Perkin-Elmer Cetus, USA) was performed at 94°C for 30 s, 55°C for 1 min and 72°C for 1 min, up to 30 cycles. Amplified DNA fragments were purified using the Sephaglas Bandprep kit (Pharmacia, Sweden).

Construction of scFv

The purified V_H and V_L DNA were assembled into a scFv fragment using a linker DNA (Pharmacia, Sweden) and the primary amplification was performed at 94°C for 1 min followed by 63°C for 4 min. The assembled scFv product was subsequently amplified with primers that create *Sfi*I and *Not*I restriction sites by using the same program as above. Following assembly, the amplified product (~750 bp) was digested with *Sfi*I and *Not*I (Pharmacia, Sweden) and purified using the Sephaglas Bandprep kit (Pharmacia, Sweden).

Cloning of the DNA fragments of V_H and V_L chains DNA into pCANTAB 5E vector

The purified scFv with *Sfi*I and *Not*I restriction sites was then cloned into a phagemid vector pCANTAB 5E (Pharmacia). Following ligation, the phagemids with the inserts were transformed into *E. coli* strain TG1. The transformed cells were plated onto SOBAG agar plate and incubated at 37°C overnight on a rocking platform at 250 rpm. After the incubation, the agar plates containing the transformants were flooded with 2X YT-AG medium and transferred to a 50-mL polypropylene tube (Falcon, USA). The cell suspension was diluted with 2X YT-AG medium to obtain an A_{600} of 0.5 and 5 mL cell suspension were used to rescue the recombinant phages.

Rescue and selection of recombinant phages expressing the scFv

Recombinant phages were rescued by adding the M13K07 helper phage (~3 x 10^{10} PFU) to the cell suspension. After incubation at 37°C for 3 h, cells were pelleted at 1000 *g* g and the pellet was resuspended in 2X YT-AK medium. The culture was incubated at 37°C for overnight on a rocking platform at 250 rpm. The culture was centrifuged as described above, and the supernatant containing the recombinant phages was used to select for scFv-expressing

clones by panning. It was performed using 25-cm^2 tissue culture flasks coated with MCF-7 cells (1 x 10^6 cells/mL) as the antigen. The coated flasks were washed 3 times with PBS and fixed by 0.06% glutaraldehyde before being blocked with PBS-B (PBS with 3% skim milk). Supernatant containing the recombinant phages was diluted with an equal volume of PBS-B and pre-incubated at room temperature for 10-15 min. After pre-incubation, the recombinant phages were added to the antigen-coated flasks and incubated at 37°C for 2 h. The flasks were washed vigorously with PBS-T (PBS with 0.05% Tween-20). After the washings, log phase *E. coli* strain TG1 in 2X YT medium was added to the flasks. Cells were incubated at 37°C for 1 h and then serially diluted tenfold dilution were carried out with 2X YT medium. An aliquot of the dilution was spread onto SOBAG agar plates and incubated at 30°C overnight.

DNA sequencing

The DNA region encoding the scFv C3A8 antibody gene was sequenced using pCANTAB 5E sequencing primer set by automated DNA sequencing systems (FIRST BASE Laboratories Sdn. Bhd, Malaysia). Single-strand DNA (ssM13 DNA) was prepared with a QIAprep M13 purification kit according to manufacturer protocol while the double-strand DNA was prepared using the PureLink™ Quick Plasmid Miniprep kit according to the manufacturer protocol.

Second rescue of recombinant phages

Well-isolated colonies on the SOBAG agar plates were transferred to separate wells of a 96-well microtiter plate (Nunc, Denmark) containing 400 mL of 2X YT-AG medium. The microtiter plate was incubated at 30°C overnight on a rocking platform at 250 rpm. The overnight culture about 40 mL was added to 2X YT-AG medium containing 1.0 x 10^8 PFU of M13K07 helper phage, and the plate was incubated at 37°C for 2 h on a shaker at 150 rpm. After the incubation, the microtiter plate was centrifuged at 1500 *g* for 20 min and the supernatant was removed. Fresh 2X YT-AK medium was added and samples were incubated overnight at 37°C with constant shaking. The bacterial cells were pelleted at 1500 *g* for 20 min and the supernatant containing the recombinant phages was removed.

Production of scFv

The supernatant consisting of the antigen-binding recombinant phages was added to log phase *E. coli* strain HB2151 in 2X YT-medium and incubated at 37°C for 30 min with intermittent gentle shaking. At the end of incubation, the culture was streaked onto a SOBAG-N plate containing 100 μg/mL naladixic acid and incubated at 30°C for overnight. A single colony of infected *E. coli* strain HB2151 on SOBAG-N was picked and grown overnight at 30°C in 2X YT-AG medium containing 100 μg/mL ampicillin and 2% glucose. The overnight culture was diluted 1:10 with fresh 2X YT-AG medium and incubated at 30°C for an hour. After incubation, the culture was centrifuged at 1500 *g* for 20 min and the supernatant was discarded. The pellet was then resuspended in 2X YT-AI medium containing 100 μg/mL ampicillin and 1 mM of isopropyl β-D-1-thiogalactopyranoside, and the cell suspension was incubated for additional 5 h. At the end of the incubation, the bacterial pellet was divided into two separate 50-mL centrifuge tubes prior to centrifugation. One pellet was resuspended in

0.5 mL of ice-cold 1X TES and the supernatant containing scFv antibody from the bacterial periplasm was collected by centrifugation. For preparation of the whole cell extract, 0.5 mL of PBS was added to the second pellet and the suspension boiled for 5 min and centrifuged. The supernatant containing scFv was collected and stored at -20°C until used.

Electrophoresis and immunoblotting

The samples were loaded into sodium dodecyl sulfate (SDS) polyacrylamide gel and run at 100 V for 90 min. The gel was stained with Coomassie blue (0.1% Coomassie blue, 40% methanol and 10% glacial acetic acid) and destained with 10% methanol and 10% glacial acetic acid buffer overnight. The protein gel was transferred to a polyvinylidene difluoride membrane (Immobilon-P, Millipore Corp., USA) for Western blot analysis using a semi-dry electroblotting system (Bio-Rad Laboratories, USA) for 45 min at 15 V in transfer buffer [25 mM Tris base, 192 mM glycine, and 20% (w/v) methanol]. The membrane was blocked with 1% bovine serum albumin in PBS for one hour and washed in 0.05% Tween 20 in PBS. The membrane was then incubated with anti E-tag horseradish peroxidase (HRP)-conjugated goat anti-mouse antibody (Amersham Pharmacia Biotech, AB) at 1:1000 at room temperature for 30 min. The conjugate was prepared in blocking buffer containing 100 mM Tris and 150 mM NaCl. The membrane was washed, and 4-chloro-1-napthol (SIGMA, USA) was then added onto the membrane. After 5 min of incubation, distilled water was added to stop the reaction and the bands were documented using Kodak film.

Detection of scFv antibody by ELISA

The indirect ELISA was carried out to determine the concentration of scFv antibodies. Initially, MCF-7 cells at a concentration of 1 x 10^6 cells/mL were seeded in 96-well plates and incubated overnight at 37°C in a 5% CO_2 incubator. The plates were washed with PBS and fixed with 0.06% glutaraldehyde before being blocked with PBS containing 2% BSA for 2h at 37°C. Each well was rinsed thrice PBS containing Tween 20 and loaded with 100 µL of scFv from both supernatant and periplasmic extract. The plain 2YT medium served as the negative control and incubated for 1h at room temperature. After rinsing three times with PBS containing Tween 20, HRP-conjugated anti-E tag antibody (Amersham Pharmacia Biotech, AB) was added. The conjugate was diluted in washing buffer at 1:100, 1:200, 1:500, 1:1000 1:2000 and 1:4000 dilutions. Finally, 100 µL of substrate solution were applied onto each well and the plates incubated for 50 min at room temperature. The plates were read at 405 nm in an ELISA auto-reader (Bio-Tek Instruments, Inc., Winooski, VT, USA).

RESULTS

Construction of single-chain fragment variable antibodies

The mRNA was isolated from the hybridoma line C3A8 and cDNA synthesis was performed. The whole cDNA reaction mixture was used for PCR amplification of the variable light and heavy chain genes. The light and heavy chains generated a sharp band at around 340 and 320 bp, respectively, as predicted. The antibody heavy and light chain DNAs were subse-

quently joined into a scFv by a $(Gly_4Ser)_3$ linker sequence. The amplified scFv was measured around 750 bp (Figure 1).

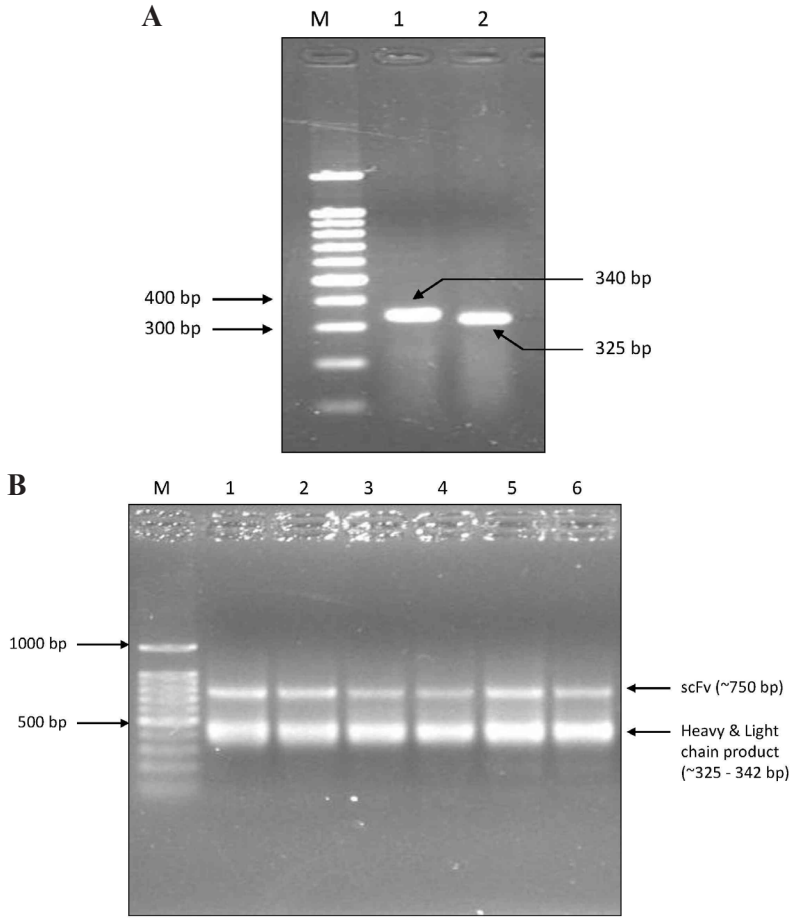

Figure 1. A. Amplification of the variable heavy (V_H) and variable light (V_L) chain genes from the cDNA of hybridoma cell line C3A8. A 3-µL aliquot of the amplified V_H (*lane 1*) and V_L (*lane 2*) products were electrophoresed on a 1.2% agarose gel in 0.5 X TAE and stained with ethidium bromide. The amplified V_H and V_L DNAs were about 340 bp and 325 bp, respectively. **B.** Amplification of the assembled single chain fragment variable (scFv). Twenty-five microliters of the amplified scFv products (*lanes 1* to *6*) were electrophoresed on a 1.0% agarose gel in 0.5X TAE and stained with ethidium bromide. The amplified scFv was around 750 bp. The DNA ladder of 100 bp was used for size indication (*lane M*).

Cloning of scFv DNA and screening of phage-displayed scFv

The amplified scFv was ligated into the pCANTAB5E expression vector. The ligated product was transformed into *E. coli* TGI and rescued by the M13K07 helper phage. The single-chain antibody was then expressed as an anchored protein on the tip of the phage particle. Random colonies of *E. coli* were picked from an SOBAG agar plate and phagemid DNA was isolated. Amplification was carried out using R1 and R2 primers to detect scFv (Figure 2).

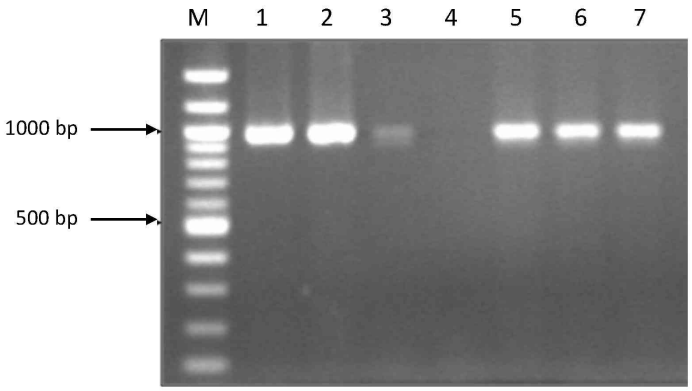

Figure 2. Detection of scFv using PCR method. Seven colonies were randomly chosen for PCR detection using R1 and R2 primers (Pharmacia, Sweeden). PCR was performed at 95°C for 5 minutes, followed by 30 cycles at 94°C for 1 minute, 55°C for 2 minutes and finally at 72°C for 2 minutes. Five microliters of each PCR products were electrophoresed on a 1% agarose gel in 0.5 X TAE and stained with ethidium bromide. *Lane M* = 100-bp DNA ladder (New England Biolabs, USA). Bands were detected in all samples with the same sizes, about 100 bp; *lane 1-2* (double stranded DNA) and *lane 3*, *5*, *6*, and *7* = single stranded DNA. *Lane 4* = Negative control where bacterial colony carrying pCANTAB 5E vector alone was used for the PCR detection.

Panning to select the phages displaying MCF-7-positive recombinant antibodies

The phage antibodies were tested for MCF-7 specific binding after the first and second rounds of panning. The phage ELISA showed an increased signal in the final round of panning (Figure 3). The phage antibody gave a very weak signal against MCF-7 before panning and increased significantly after a number of rounds of panning (data not shown). It has been demonstrated that repeating biopanning enriches bacteriophages carrying the scFv sequences. After panning, 48 antigen-positive phage clones were randomly picked and screened by ELISA. The 4 clones D11, B7, B1 and A10 showed the strongest signal, and one out of the 4 clones, clone B7, was selected for further study (Table 1). Following selection, the strongest positive clone B7 was used to compare antigen binding reactivity with the Mab derived from the C3A8 parental hybridoma. It showed that C3A8 scFv could retain the binding specificity of the parent Mab, since the ratio for the parent Mab was 7.577, while 5.625 for C3A8 scFv (Table 2).

DNA sequence analysis

The phagemid derived from clone B7 was used for DNA sequencing, and the results showed that the V_H and V_L DNAs of scFv DNA were about 340 bp and 320 bp, respectively (Figure 4). The CDR regions in V_H and V_L amino acids were determined using KABAT and Chothia numbering as represented in Figure 5.

Expression of soluble antibody from cloned scFv fragments

E. coli HB2151 cells were infected with strong positive phage obtained after two rounds of panning. The amber stop codon was recognized between the scFv and the M13 gene III pro-

tein, resulting in the production of soluble scFv by *E. coli.* The soluble scFv were directed to the periplasmic space by an NH2-terminal gene III protein leader sequence. The peptide epitope E-tag incorporation at the COOH-terminal allowed the detection of anti-E-tag antibody. Upon induction with isopropyl-D-thiogalactopyranoside (IPTG), the culture extracts were subjected to SDS-PAGE and Western blotting. An immunoreactive band with an apparent molecular mass of about 32 kDa was detected in the periplasmic and supernatant extracts (Figure 6). The scFv recombinant protein was not observed in the noninduced culture (Figure 7).

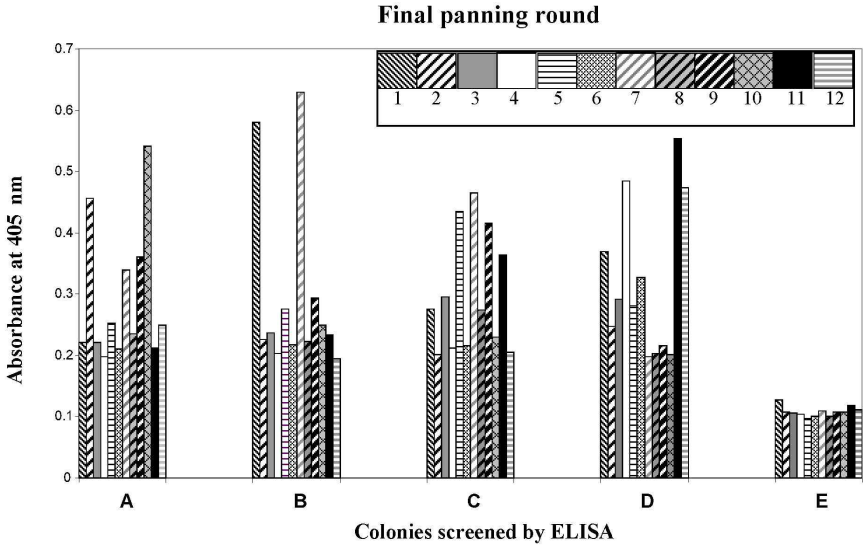

Figure 3. Performance of 48 preselected positive clones (columns A to D) and negative control (column E) were screened for binding activity against MCF-7 by phage ELISA. Each column represents the ELISA signal from an individual clone isolated after second round of biopanning. Four clones (A10, B1, B7, and D11) that showed the highest representative were selected for further study.

Table 1. Reactivity of scFv antibodies against MCF-7.

ELISA	scFv antibodies		Negative control
OD	From supernatant (after 20 X concentrated)	From periplasmic extract	
	0.706	0.480	0.106

Cell ELISA results of the scFv antibodies (derived from periplasmic and supernatant extracts) against MCF-7 that was expressed by positive phage recombinants in *Escherichia coli* strain HB2151.

Table 2. Screening for strongest phage recombinants displaying scFv antibodies.

ELISA	No. of strong positive clones				Negative control
OD	1 (D11)	2 (B7)	3 (B1)	4 (A10)	
	0.554	0.630	0.580	0.541	0.112

Screening for phage positive clones displaying antigen binding antibodies against MCF-7 cells before production of soluble antibodies in *Escherichia coli* HB2151. All four clones, which showed the highest absorbance, were set aside for further study.

```
gi|16124255|gb|AAG28706.2|   single chain antibody against rice stripe virus
protein P20 [synthetic construct]
Length=261

 Score =  434 bits (1115),  Expect = 2e-120
 Identities = 241/243 (99%), Positives = 241/243 (99%), Gaps = 0/243 (0%)
 Frame = +1

Query  1    MAQVKLQQGTEVVKPGASVKLSCKASGYIFTSYDIDWVRQTPEQGLEWIGWIFPGEGST   180
            MAQVKLQQGTEVVKPGASVKLSCKASGYIFTSYDIDWVRQTPEQGLEWIGWIFPGEGST
Sbjct  1    MAQVKLQQGTEVVKPGASVKLSCKASGYIFTSYDIDWVRQTPEQGLEWIGWIFPGEGST    60

Query  181  EYNEKFKGRATLSVDKSSSTAYMELTRLTSEDSAVYFCARGDYYRRYFDLWGQGTTVTVS  360
            EYNEKFKGRATLSVDKSSSTAYMELTRLTSEDSAVYFCARGDYYRRYFDLWGQGTTVTVS
Sbjct  61   EYNEKFKGRATLSVDKSSSTAYMELTRLTSEDSAVYFCARGDYYRRYFDLWGQGTTVTVS  120

Query  361  Scgggsggggsggggs DIELTQSPAIMSASPGERVTMTCSASSSIRYIYWYQQKPGSSPR  540
            SCGGGS GGGSGGGGSDIELTQSPAIMSASPGERVTMTCSASSSIRYIYWYQQKPGSSPR
Sbjct  121  SCGGGSSGGGSGGGGSDIELTQSPAIMSASPGERVTMTCSASSSIRYIYWYQQKPGSSPR  180

Query  541  LLIYDTSNVAPGVPFRFSGSGSGTSYSLTINRMEAEDAATYYCQEWSGYPYTFGGGTKLE  720
            LLIYDTSNVAPGVPFRFSGSGSGTSYSLTINRMEAEDAATYYCQEWSGYPYTFGGGTKLE
Sbjct  181  LLIYDTSNVAPGVPFRFSGSGSGTSYSLTINRMEAEDAATYYCQEWSGYPYTFGGGTKLE  240

Query  721  LKR  729
            LKR
Sbjct  241  LKR  243
```

Figure 4. BLAST-P search hit for anti-MCF7 scFv against PDB (protein databank). The results show that the anti-MCF7 is similar to a single chain antibody against rice stripe virus protein P20 (synthetic construct) at a similarity of 99% (gi|16124255|gb|AAG28706.2|).

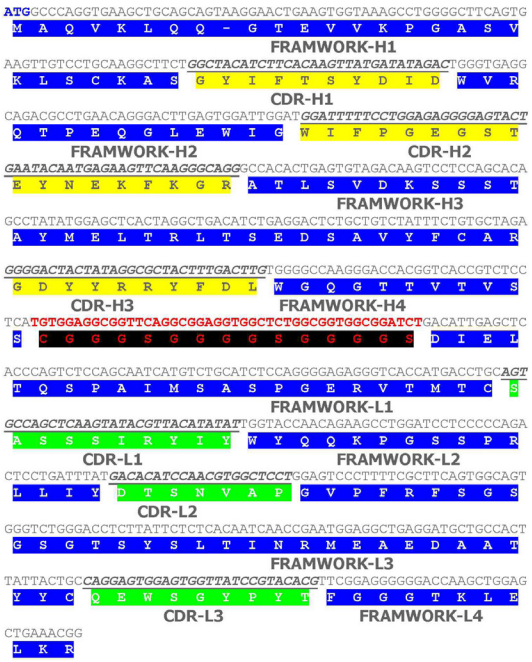

Figure 5. DNA and protein sequences for V_H and V_L chains. Both V_H and V_L chains were obtained from FIRST BASE Laboratories Sdn. Bhd and the TRANSLATE program is used to translate into protein sequences. The 3 CDRs for both the chains were obtained using KABAT numbering and the CDR sequences are shown in yellow and green highlight**.**

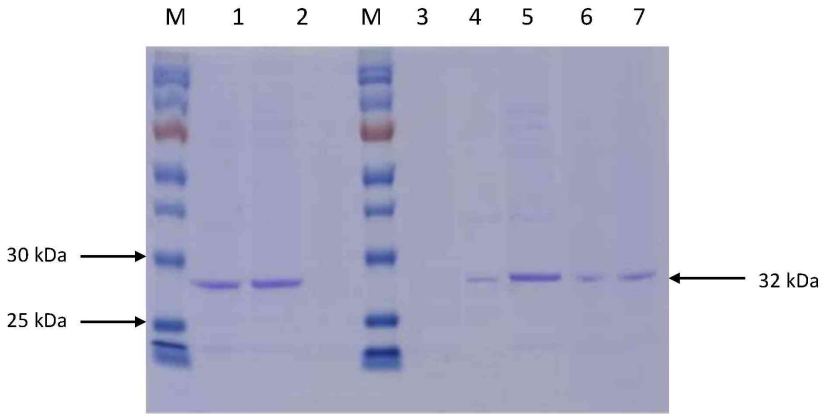

Figure 6. SDS-PAGE analysis for the detection of soluble C3A8 scFv antibodies from periplasmic and supernatant extracts. *Lanes 1* and *2* = periplasmic extracts. *Lane 3* = extract from negative clone; pCANTAB 5E phagemid vector without insert (blank). *Lanes 4* to *7* = supernatant extracts. *Lane M* = PageRuler™ Prestained Protein Ladder (Fermentas).

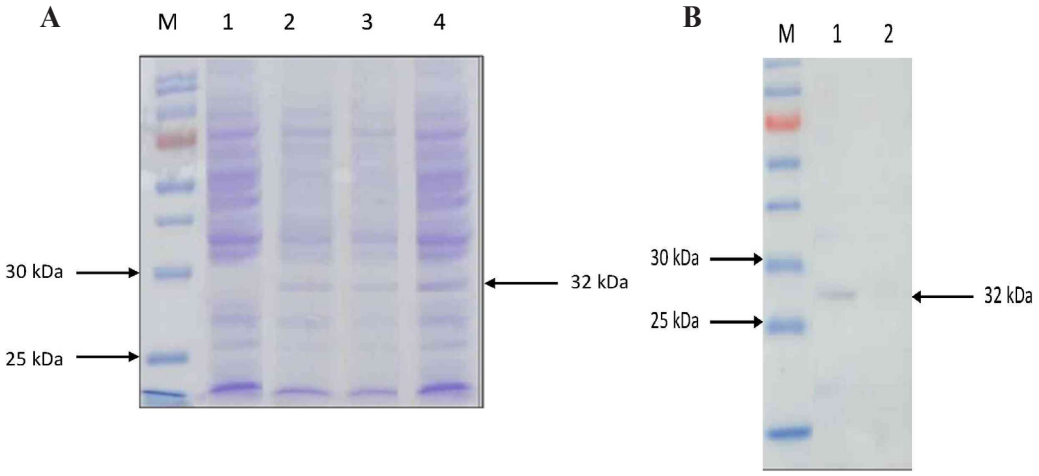

Figure 7. A. *Lane 1* = crude extract from negative clone; pCANTAB 5E phagemid vector without insert (blank). *Lanes 2* to *4* = SDS-PAGE for detection of insoluble C3A8 scFv from crude cells extract. **B.** *Lane 1* = Western blotting of scFv antibody detected with HRP/anti-E tag antibody. Inserts were grown in the expression vector pCANTAB 5E. One milliliter induced culture was centrifuged at 10 000 *g* for 3 min and resuspended in 100 mL of 2 X sample buffer prior to preheat at 95°C for 5 min. Next, the denatured protein separated by SDS-PAGE before being transferred onto PVDF membrane. The membrane then probed using HRP/anti-E tag antibody and finally developed using 4-CN substrate. A 32-kDa band was visualized on the membrane as anticipated. *Lane M* = PageRuler™ Prestained Protein Ladder (Fermentas).

Quantification of the scFv fragment specificity against MCF-7 by indirect ELISA

After the expression of soluble scFv in *E. coli* HB2151, all three different fractions were analyzed by ELISA and Western blotting to confirm the presence and reactivity of scFv.

The results showed that scFv obtained from the supernatant and periplasmic extract expressed high binding activity towards MCF-7, whereas no significant binding signal was obtained with whole-cell extracts.

DISCUSSION

The emerging technology of recombinant antibodies has brought new promising approaches towards expression of antibody fragments using phage display system in the field of cancer therapy because of its wide advantages over hybridoma technology, such as generating high-affinity antibodies with increased specificity, and involving quick and economical procedures (Shadidi and Sioud, 2001; Govorko et al., 2001). In the present study, we constructed the scFv antibodies using hybridoma cells through phage display technology. Normally, the concentration and purity of mRNA derived from C3A8 hybridoma are crucial for sufficient gene amplification of heavy and light chain. The amplified V_H and V_L DNAs measured about 340 bp and 320 bp respectively. These anticipated molecular weights were confirmed by DNA sequencing of scFv genes. According to DNA sequencing, the amino acids of CDR regions in V_H and V_L were determined using KABAT numbering. It is important that the concentration of the two DNA chains be the same to construct a successful scFv. Therefore, we compared the intensity of bands with the known quantities on a DNA ladder (data not shown). This concentration was then employed in the subsequent PCR, which yielded the single-chain variable fragment DNA of 750 bp. More than one assembly PCR was performed to obtain a sufficient amount of pure scFv DNA.

The amplified V_H and V_L genes were joined with a neutral linker and expressed as a single polypeptide chain; however, the joining of the V_H and V_L genes together with the peptide linker enables both of the regions to associate intra-molecularly and stabilizes variable domain combinations that react weakly (Krebber et al., 1997). Therefore, the flexible peptide linker consisting of 15-amino acid $(Gly_4Ser)_3$ was used to bridge the 3.5 nm gap between the carboxy-terminus of the V_H chain and the amino terminus of the V_H and V_L chain. This construction facilitated the chain pairing and minimized the refolding and aggregation problems encountered (Whitlow et al., 1991). Apparently, the affinity and stability of scFv antibodies with $(Gly_4Ser)_3$ were similar to native antibodies (Takkinen et al., 1991). In our study, we demonstrated that the engineered C3A8 scFv could preserve the reactivity of their parental Mab.

The single chain antibody was expressed on the tip of the filamentous phage as a membrane-anchored protein. This provided antibody selection in a very efficient way as well as isolating the best candidate for scFv antibodies. We determined the best four candidates from our sample, and the selected positive phage recombinants were used to infect expression host *E. coli* HB2151. The phage antibodies showed high specificities against MCF-7 either as a component of the phage or soluble antibodies. We incorporated pCANTAB 5E as a g3p leader sequence that facilitated the direct transport of the scFv to the inner membrane of *E. coli*. In concordance with other studies, we demonstrated that the soluble scFv antibodies accumulated in the periplasm of *E. coli* HB2151 upon extended incubation (Hoogenboom et al., 1991). The scFv antibodies were visualized as a 32 kDa band, and this indicated that most scfv antibodies were produced by the clones as inclusions or insoluble forms. The inclusion bodies were harvested and the aggregated proteins were solubilized with 8 M urea to obtain the active scFv (Cao et al., 2005). In the present study, we showed an efficient means of obtaining large amounts of scFv.

In summary, recent advances in antibody engineering such as phage display technology can be used to develop clinically relevant anti-MCF-7 antibody fragment to achieve optimal tumor localization and also to serve as a targeting vehicle for therapeutic applications in breast cancer.

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