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Molecular Structural and Functional Characterization of Tumor Suppressive Anti–ErbB-2 Monoclonal Antibody by Phage Display System

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To investigate the molecular structural and functional characteristics of tumor-suppressive anti–ErbB-2 monoclonal antibody (mAb) SER4, we performed mAb-gene cloning and epitope mapping by a phage display system. Structural analysis demonstrated that both the heavy chain (HC) and light chain variable regions are highly homologous with the derived germline sequences, while the HC complementarity determining region (HCDR) 3 has a relatively short length and biased amino acid usage. A cloned gene-derived recombinant Fab (rFab) fragment showed antigen binding activity and specificity comparable to the parent mAb. Cross-linking of the rFab fragment with the anti-Fab antibody elicited cell growth inhibition *in vitro*. These results imply that the cloned genes actually encode the Fab part of SER4. The epitope mimetic peptide (mimotope) isolated by panning a phage-displayed random peptide library against SER4 showed no cross-reactivity with mAbs other than SER4. The mimotope was found to be homologous with ^{87}AHNQVRQVPLQR^{98} in the extracellular domain of ErbB-2 by means of a clustalw search. Since SER4 causes the growth inhibition of ErbB-2 positive cells, the predicted epitope sequence may constitute the putative functional domain of ErbB-2.

Key words: epitope mapping, ErbB-2, monoclonal antibody, phage display, recombinant Fab.

Abbreviations: CDR, complementarity determining region; ELISA, enzyme-linked immunosorbent assay; NRS, normal rabbit serum; SB, super broth.

Clinical applications of monoclonal antibodies (mAbs) for diagnosis and therapy in human cancer have been a very active area of research. MAbs alone or conjugated to isotopes, toxins, or anti-cancer drugs have been employed in clinical trials. Cell surface antigens that are expressed dominantly on tumor cells are preferable targets for antibody tumor therapy. ErbB-2, the product of the *c-erbB-2* protooncogene, is a transmembrane tyrosine kinase found to be overexpressed on certain tumors, whereas it is rarely expressed on adult human tissues. For instance, ErbB-2 is highly expressed on 20–30% of breast cancers (*1*, *2*), and this overexpression is correlated with a poor prognosis in patients with these tumors (*1*, *3*). A number of mouse and rat mAbs have been prepared against the extracellular domain of ErbB-2 (*4*, *5*). Transtumab (Herceptin), a humanized mAb of mouse anti–ErbB-2, 4D5 (*6*), is now clinically used to treat metastatic breast tumors that overexpress ErbB-2. Transtumab itself has an inhibitory effect on tumor growth, and also the co-administration of Transtumab with anti-tumor agents, such as cisplatin or paclitaxel, shows a synergistic effect on tumor growth suppression in clinical studies (*7*).

Small fragments with antigen binding activity, such as recombinant Fab (rFab) fragments, would be advantageous as compared with the mAb with respect to their lower immunogenicity and better pharmacokinetic properties (*8*). rFab-toxin complexes can be produced easily by connecting the genes of Fab and toxins followed by expression in *E. coli* as recombinant fusion proteins.

Antibody phage display (*9*, *10*) is a recently developed recombinant DNA technology for preparing recombinant antibody fragments from various immune sources, such as bone marrow cells, lymph nodes, peripheral blood lymphocytes, spleen cells, and hybridoma cells. rFab fragments against viral pathogens (*11–14*), autoantigens (*15–18*), and tumor-associated antigens (*19–22*) have been successfully prepared by phage display systems. Phage display systems have also been applied to epitope mapping of the mAbs of interest (*23*).

In this study, to understand the structure and function relationship of tumor-suppressive anti–ErbB-2 mAb SER4 (*24*) and to develop SER4-derived novel tumor therapeutic agents, we performed mAb gene cloning,

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characterization of the rFab fragment, and epitope mapping by means of a phage display system. The findings indicate that the heavy chain complementarity determining region (HCDR) 3 shows characteristic length and amino acid usage, that the expressed rFab fragment shows reactivity and specificity comparable to the parent mAb, and that the isolated epitope mimetic peptide (mimotope) sequence shows homology with the extracellular domain of ErbB-2.

MATERIALS AND METHODS

Cell Culture—SER4 (IgG_1, κ)-producing hybridoma cells were cultured in RPMI1640 medium supplemented with 100 U/ml of penicillin G, 100 μg/ml of streptomycin, 2 mM glutamine, and 10% heat-inactivated fetal bovine serum (FBS). Human carcinoma (c.a.) cell lines, $HeLaS_3$ (cervix c.a.), T24 (bladder c.a.), MDA-MB-453 (breast c.a.), MCF-7 (gastric c.a.), LS-LM4 (colon c.a.), Int407 human fetal kidney, ACHN (renal c.a.), KNS human glioma and SV-T2 (SV40-transformed BALB/3T3 mouse fibroblastic cells) were cultured in minimum essential medium (MEM) with the additives mentioned above. Peer human leukemic cells were cultured in the medium used for hybridoma culture. All cell lines were maintained at 37°C in a humidified 5% CO2 atmosphere.

Library Construction and Phage Selection—A Fab phage display combinatorial library was constructed from SER4-producing hybridoma cells as described previously (*19*, *22*). The constructed library was electroporated into XL1-Blue cells (Stratagene, La Jolla, CA) by an E.coli pulser (Bio-Rad, Richmond, CA), then streaked onto LB agar plates containing 100 μg/ml of carbenicillin and 100 mM glucose. Single colonies were cultured in Super broth (SB) with 50 μg/ml of carbenicillin, and Fab-displayed phage (Fab-phage) clones were rescued by infection with VCSM13 helper phage (Stratagene). The amplified Fab-phage clones were tested for binding activity to formalin-fixed MDA-MB-453 cells by an indirect immunofluorescence assay as described below. The identity of the positive clones was determined by *Bst*NI fingerprinting (*16*).

Nucleic Acid Sequencing—Nucleic acid sequencing was carried out using a PRISM 310 genetic analyzer (ABI, Foster City, CA) with a dye terminator cycle sequencing FS ready reaction kit (ABI). The primers SeqT3 (5′-ATT AAC CCT CAC TAA AG-3′) and KEF (5′-GAA TTC TAA ACT AGC TAG TTC G-3′), both hybridizing to the (–)-strand, were used for sequencing the V_H and V_L domains, respectively. Comparison with the reported immunoglobulin germline sequences from Genbank/EMBL/DDBJ was performed by Ig BLAST analysis and by DNAPLOT analysis.

Production and Purification of rFab Fragment—Reconstruction of a phagemid DNA and production of the rFab fragment from *E. coli* was performed as described previously (*11*, *25*). In brief, single *E. coli* colonies bearing the phagemid DNA reconstructed for rFab production were inoculated into 100 ml of SB with 50 μg/ml of carbenicillin and 20 mM $MgCl_2$ and cultured for 6 h at 37°C with vigorous shaking. Protein expression was induced with 1 mM isopropyl β-D-thiogalactopyranoside (Roche Diagnostics) and the cells were incubated overnight at 30°C. The rFab fragment was purified from the supernatant of bacterial lysates by Ni-chelate chromatography using a Ni-NTA spin kit (Qiagen) as described previously (*22*).

Immunological Assays—The reactivity with and specificity to ErbB-2-positive and -negative cells of the rFab fragment were determined by an indirect immunofluorescence assay. Sub-confluent cultures of MDA-MB-453 (ErbB-2^+) and $HeLaS_3$ (ErbB-2^-) cells on Lab-Tek chamber slides (Nalgenunc International, Tokyo) were fixed with 4% (v/v) formalin-PBS. For unfixed cell staining, MDA-MB-453 and $HeLaS_3$ cells were detached from the culture flask with trypsin-EDTA (GIBCO-BRL, Lifetech Oriental, Tokyo), then washed once with PBS, and suspended in 100 μl of PBS (1×10^5 in each assay). An isotype matched rFab fragment against verotoxin-1 (manuscript in preparation) was used as a negative control for testing the specificity of the rFab fragment. The fixed or unfixed cells were successively treated with 5% (v/v) normal rabbit serum (NRS) (Jackson ImmunoResearch, West Grove, PA) in PBS (NRS-PBS), rFab fragment (10 μg/ml in NRS-PBS), and FITC-labeled rabbit anti-mouse IgG $F(ab')_2$ (Jackson ImmunoResearch) (1:300 diluted in NRS-PBS). The samples were examined with a confocal laser microscope (LSM410, Carl Zeiss Japan, Tokyo).

The reactivity of the rFab fragment with a panel of cultured human cell lines (see the cell culture section) was determined by flow cytometry. Cells in suspension were successively treated on ice with each rFab fragment (10 μg/ml in NRS-PBS), and FITC-labeled rabbit anti-mouse IgG $F(ab')_2$ (1:300 diluted in NRS-PBS). The mean fluorescence intensity (MFI) of individual cells was determined with a FACScan flow cytometer (Becton Dickinson, Sunnyvale, CA).

Cell Growth Inhibition Assay—The cell growth inhibitory activity of the rFab fragment was tested using MDA-MB-453 and $HeLaS_3$ cells. The cells were seeded in triplicate into flat-bottomed 96-well culture plates at 1×10^4 cells per well. The rFab fragment alone or the rFab fragment cross-linked with the rabbit anti-mouse IgG $F(ab')_2$ was incubated with cells for 72 h at 37°C. The rFab fragment was used at a final concentration of 50 μg/ml, and the same concentration of mAb was used as a positive control. The cell growth inhibitory effect of the rFab fragments was evaluated by the Alamar Blue (Cosmo Bio, Tokyo) assay according to the manufacturer's instructions.

Epitope Mapping Using a Phage Displayed Random Peptide Library—The dodecamer peptide library displayed on the gene III product of M13 phage (PhD-12, New England BioLab, Beverly, MA) was used for epitope mapping of SER4. SER4 (10 μg/well) was immobilized on two wells of a Costar 3590 microtiter plate (Corning International, Tokyo). After overnight incubation at 4°C, the solution was discarded and the wells were blocked with Blockace (Dai-nippon Pharmacy, Osaka) for 1 h at 37°C. After discarding the blocking solution, phage library solution (10^{13} pfu/well) was added to the wells, and the plates were incubated for 2 h at 37°C. After the wells were washed 10 times with 0.05% Tween 20-PBS (T-PBS) with vigorous pipetting, the bound phages were eluted with 0.2 M glycine-hydrochloride (pH 2.2). The eluate was immediately neutralized with 2 M Tris, then

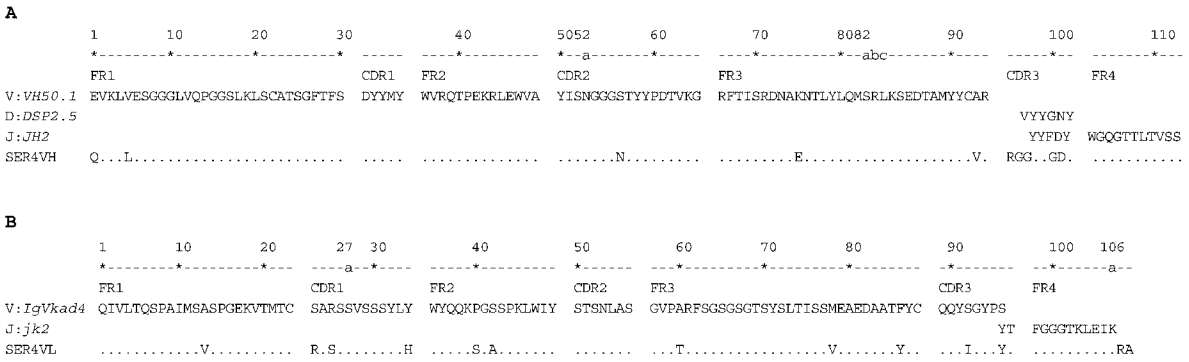

Fig. 1. **Deduced amino acid sequences of the V_H and V_L domains of SER4 with the closest known germline.** (A) The SER4 V_H sequence was compared with the closest germline V segment (*VH50.1*), D segment (*DSP2.5*), and J segment (*JH2*). (B) The SER4 V_L sequence was compared with the closest germline V segment (*IgVkad4*) and J segment (*jk2*). Standard Kabat numbering (*42*) is shown above the sequence.

infected with a mid-log culture of XL1-Blue. Phage amplification was continued for 4.5 h with vigorous shaking, after which the supernatant was collected by centrifugation and precipitated with PEG/NaCl overnight at 4°C. The PEG-precipitated phage was dissolved in PBS, then titered and used for the second round of panning. Phage clones obtained after the third round of panning were tested for binding activity to SER4 using a horseradish peroxidase-labeled rabbit anti-M13 antibody (Amersham Biosciences, Tokyo) (1:1,000 diluted in T-PBS). The M13 phage single-stranded DNA was prepared from positive clones with a QIAspin M13 Kit (Qiagen, Tokyo). Nucleic acid sequencing using the –96 gIII sequencing primer (5′-CCC TCA TAG TTA GCG TAA CG-3′) was performed as described in the nucleic acid sequencing section. Comparison to the ErbB-2 extracellular domain sequence was performed by the clustalw search.

RESULTS

Phage Display cDNA Cloning and Characterization of SER4 Genes—The antibody heavy chain (HC) Fd and light chain (LC) genes were amplified by PCR using cDNA synthesized from total RNA of SER4-producing hybridoma cells. The amplified products were pooled, gel-purified, and digested with restriction enzymes corresponding to the cloning sites. The constructed IgG_1, κ antibody combinatorial library contained 3×10^6 transformants. Fifty single colonies from the library were separately cultured, then Fab-displayed phage clones rescued by infection with helper phage were tested for binding to formalin-fixed MDA-MB-453 cells by an indirect immunofluorescence assay. All positive clones (12/50) were identical by *Bst*NI fingerprinting analysis (data not shown).

To elucidate the molecular structural characteristics of SER4, the V_H and V_L domains of the selected clone were sequenced and analyzed. The deduced amino acid sequences together with the derived germline genes are shown in Fig. 1. The V_H of SER4 was found to be a member of the mouse V_H5 family, while the LC belongs to the mouse Vk4/5 group IV subgroup. Ig BLAST analysis and DNAPLOT analysis revealed that SER4 is derived from the germline genes of $V_H50.1$ (94.9% amino acid homology) and IgVkad4 (88.7% amino acid homology), respectively. The highly conserved germline sequence in SER4 V_H and V_L suggest that somatic mutations have rarely occurred in the V segments of SER4, while the HCDR3 sequence has the characteristics of a relatively short length and high frequency of glycine and tyrosine residues. Although further studies, such as point mutation studies, should be performed, these results imply that HCDR3 may be the major determinant of antigen recognition by SER4.

Expression and Characterization of the SER4 rFab Fragment—The rFab fragment produced from the clone reengineered for rFab expression was recovered from the periplasm of IPTG-induced packed cells by freeze-thawing, and then affinity-purified by Ni-chelate chromatography. A single band of approximately 50-kDa with definitive antigen binding activity was detected in the 250 mM imidazole eluate fraction by SDS-PAGE after Coomassie Brilliant Blue staining (data not shown). The optically determined yield of the purified rFab fragment was 27 μg from 100 ml of culture.

The antigen binding activity and specificity of the rFab fragment were evaluated by means of an indirect immunofluorescence assay. The rFab fragment reacted mainly with the cell membrane fraction rather than the intracellular fraction of fixed MDA-MB-453 cells (Fig. 2A). The staining pattern of the corresponding unfixed cells showed that the rFab fragment could bind to the surface of living cells (Fig. 2B). The binding of the rFab fragment to MDA-MB-453 cells is an ErbB-2–specific reaction since the rFab fragment showed no reactivity with ErbB-2–negative $HeLaS_3$ cells (Fig. 2C) and an isotype-matched rFab fragment directed to the verotoxin-1 showed no reactivity with MDA-MB-453 cells (Fig. 2D).

The reactivity of the rFab fragment against a panel of human cultured cell lines was determined by flow cytometry (Fig. 3). The rFab fragment reacted only with cell lines expressing ErbB-2 on their cell surfaces (MDA-MB-453, MCF-7, LS-LM4).

SER4 mAb shows growth-inhibitory activity against ErbB-2-expressing cells *in vitro* (*24*). The rFab fragment was examined for its ability to inhibit cell growth using antigen-positive MDA-MB-453 and antigen-negative

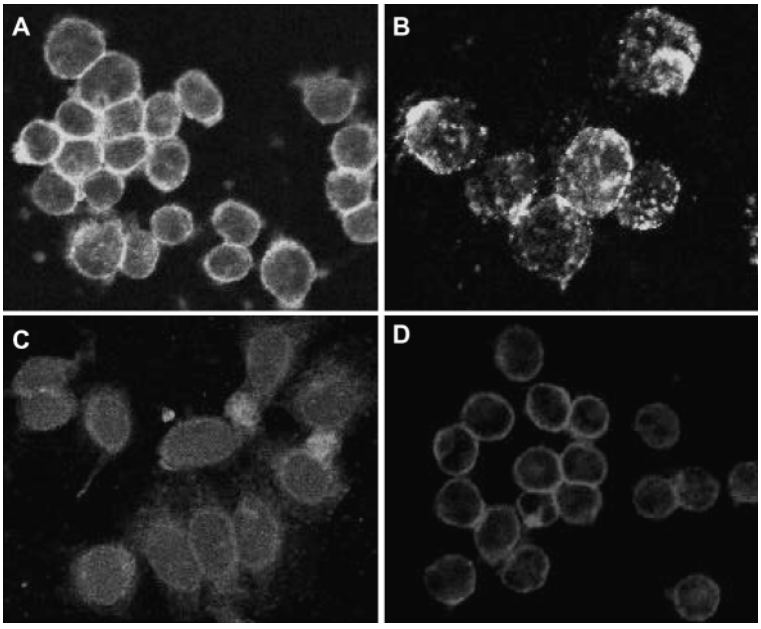

Fig. 2. **Indirect immunofluorescence staining with the SER4 rFab fragment of ErbB-2-positive and -negative cultured cell lines.** A and B, fixed and unfixed MDA-MB-453 cells (ErbB-2$^+$) stained with the rFab fragment; C, fixed HeLaS_3 cells (ErbB-2$^-$) stained with the rFab fragment; D, MDA-MB-453 cells stained with anti-verotoxin-1 rFab fragment.

HeLaS_3 cells. Although the rFab fragment alone had no growth inhibitory activity against antigen-positive MDA-MB-453 cells, the rFab fragment cross-linked to rabbit anti-mouse IgG F(ab′)$_2$ inhibited cell growth to the same extent as the whole mAb (Fig. 4). Cell growth inhibition by the cross-linked rFab fragment is antigen-specific since it showed no growth inhibition of antigen-negative HeLaS_3 cells. Binding of the SER4 mAb to the epitope followed by cross-linking of the antigen is indispensable for eliciting the cell growth-inhibitory activity. These results imply that the rFab fragment produced by the cloned gene-bearing *E. coli* was actually identical to the Fab part of the SER4 mAb.

Epitope Mapping of SER4 Using a Phage Displayed Random Peptide Library—To identify the epitope sequence recognized by SER4, we performed panning of a phage-displayed random peptide library expressing the 12-mer amino acids against the plate-coated SER4. The eluted phage number was increased from 9.3×10^4 pfu (after the first round of panning) to 1.3×10^8 pfu (after the fourth round of panning). An approximately 1,400-fold enrichment of the library was observed in the course of panning. We picked up a total of 120 plaques from the plate obtained after the final round of panning, and the amplified phage clones were tested for binding activity to SER4 by an enzyme-linked immunosorbent assay (ELISA). As a result, 21 clones were found to be positive against SER4 with no cross-reactivity to the isotype-matched anti-CD98 heavy chain mAb, HBJ127 (*26*). Nucleotide sequencing of the ELISA-positive clones demonstrated that all clones had an identical sequence: INNEYVESPLYM. A representative clone, S307, showed the reactivity to SER4 in a concentration dependent manner with no cross-reactivity to the different ErbB-2 epitope-directed mAb, SER8 (Fig. 5). A clustalw search demonstrated that the isolated epitope mimetic peptide (mimotope) was homologous to the region ^{87}AHNQVRQVPLQR^{98} in the extracellular domain of ErbB-2 (Fig. 6). Since SER4 is a tumor-suppressive mAb, the predicted epitope region may constitute the putative functional domain of ErbB-2. Detailed analysis of the SER4-recognizing epitope using the isolated mimotope peptide is now in progress.

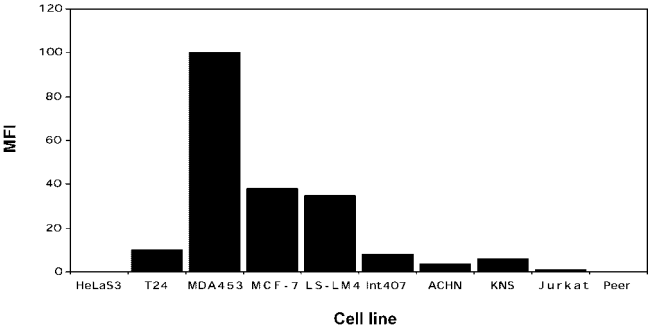

Fig. 3. **Reactivity of the SER4 rFab fragment against a panel of cultured human cell lines.** Unfixed cells were reacted with the rFab fragment, and then analyzed for mean fluorescence intensity (MFI) by FACScan flow cytometer.

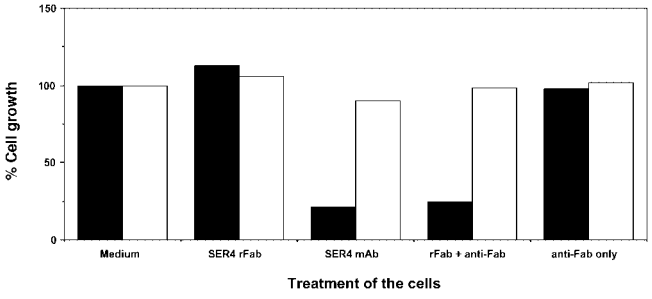

Fig. 4. **Effect of the SER4 rFab fragment on *in vitro* cell growth of MDA-MB-453 and HeLaS_3 cells.** Cells (1×10^4) were cultured with the rFab fragment (SER4 rFab), mAb (SER4 mAb), rFab fragment cross-linked with rabbit anti-mouse IgG F(ab′)$_2$ (rFab + anti-Fab), or rabbit anti-mouse IgG F(ab′)$_2$ (anti-Fab only) for 72 h at 37°C. Cell growth was then evaluated by Alamar Blue assay. Solid square, MDA-MB-453; open square, HeLaS3

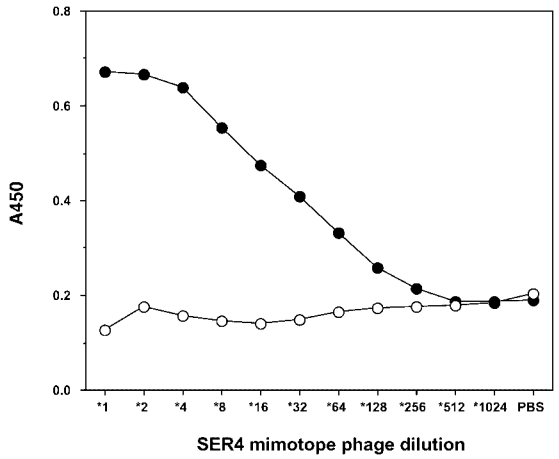

Fig. 5. **Reactivity of a SER4-mimotope-bearing phage clone against SER4 and SER8.** The mimotope phage (initial phage titer: 5.3×10^{12} pfu/ml) was diluted twofold and reacted with SER4 or SER8. Bound phages were detected by HRP-labeled rabbit anti-M13. Solid circle, SER4; open circle, SER8.

HER2-ECD: ^{87}AHNQVRQVPLQR^{98}

Mimotope: INNEYVESPLYM

Fig. 6. **Prediction of the SER4-recognizing epitope in the extracellular domain of ErbB-2.** The homology between the mimotope sequence and the extracellular domain of ErbB-2 (HER2-ECD) was performed by clustalw search. Identical amino acids are shown in bold, and conservative substitutions are underlined.

DISCUSSION

In this study, we utilized phage display methodology for the molecular structural and functional characterization of tumor-suppressive anti–ErbB-2 mAb SER4. Antibody phage display was used to clone mAb genes, and a phage-displayed random peptide library was used for epitope mapping of the mAb. As a result, we defined HCDR3 as a putative determinant of antigen recognition by SER4, and found that a mimotope sequence homologous region is located in the extracellular domain of the ErbB-2 oncoprotein.

Recently, antibody phage display has become a general strategy for preparing recombinant antibody fragments of human (*11–18*), mouse (*19*, *20*, *22*) and other species origin (*27*, *28*). Antibody genes can be expressed as Fab or single chain Fv (scFv) using suitable vectors and cloning systems. We used the pComb3 system first reported by Barbas, *et al.* (*25*). This system is optimized for the expression of antibody genes as the Fab form and for targeting recombinant proteins to the periplasmic space of host cells by the pelB leader sequence. The HC Fd and LC fragments are thought to associate through the formation of a disulfide-bond in the periplasmic space under reducing conditions. rFab fragments that accumulate into the periplasmic space can easily be recovered by sonication or freeze-thawing the host cells with a minimum loss of antigen binding activity. A commercially available secondary antibody directed to IgG $F(ab')_2$ can be used to detect antigen-bound rFab fragments. The pComb3 system used in this study is, therefore, better suited to the cloning and expression of mAb genes than the other systems.

The V_H domain of an antibody is known to play a major role in antigen recognition and binding (*14*, *29*). Somatic mutations in V segments during B cell maturation are indispensable for rearranged antibodies to obtain high specificity and affinity (*30*, *31*). HCDR3 located at the junction of V-D-J segments is also thought to be important for antigen recognition because of its diversity in length and amino acid usage (*32*). Sequence analysis of cloned genes revealed that the V_H domain is 94.9% homologous to derived germline V_H50.1 and the V_L is 88.7% homologous to IgVkad4. This implies that somatic mutations occur rarely in the V segments of HC and LC during B cell maturation. On the other hand, the characteristic features of relatively short length (8 amino acids) and biased amino acid usage (glycine and tyrosine) were observed in the HCDR3 sequence. The importance of antibody CDR residues in antigen recognition has been reported. Schier *et al.* demonstrated that the introduction of mutations into both the heavy- and light-chain CDR3 of anti-ErbB-2 scFv yielded an approximately 1,000-fold increase in affinity from that of the original scFv (*33*). Analysis of the antigen-binding site of an anti-ErbB-2 antibody by means of several mutation studies has also been reported. Vajdos *et al*. presented the comprehensive functional maps of the antigen binding site of an ErbB-2 antibody by shotgun scanning mutagenesis of all six CDR residues (*34*). Gerstner *et al.* identified the plastic site in a therapeutic anti–ErbB-2 antibody (hu4D5) by substitution of amino acid residues with all 20–amino acids in selected CDR positions and one framework position (*35*). Although similar mutation studies as above are needed to confirm our findings, the results suggest that HCDR3 is a promising candidate for the antigen binding site of SER4. Furthermore, the HCDR3 sequence may be applicable to preparing novel anti-tumor agents such as CDR peptides (*36*), small peptides with antigen binding activity.

We demonstrated that the rFab fragment produced by the phage display system has affinity and specificity almost equal to those of parent mAb. The minimum loss of antigen binding activity during expression in *E. coli*, recovery from cells, and purification by Ni-chelate chromatography would be expected. We also demonstrated that the rFab fragment cross-linked with anti-Fab antibody inhibits cell growth. This preliminary experiment shows the possible use of SER4 rFab genetically fused to the human IgG Fc cassette (*37*) for cancer immunotherapy. We can also prepare the rFab-toxin fusion protein as an effective cancer therapeutic. For clinical applications of mAb, human antibodies against tumor antigens would be the best although they are very difficult to isolate because of the low immunogenicity of tumor antigens in humans. Figini *et al.* reported the isolation of a human antibody against the folate-binding protein (FBP), a cell surface antigen that is overexpressed in many human ovarian carcinomas, using the genes of high affinity mouse mAb against FBP as guides for selection, a technique termed guided selection (*38*). The SER4 mAb genes cloned in this study would be applicable as guides for the isolation of the human rFab fragment against ErbB-2.

Epitope mapping has been applied to understanding molecular mechanisms in several systems by defining the precise residues present in structurally or functionally important epitopes (*39*). We utilized phage display epitope analysis for the tumor-suppressive antibody, SER4, which recognizes the extracellular domain of ErbB-2. By combining this information with the cell growth inhibitory effects of SER4, a novel structure-function relationship of ErbB-2 can be considered. Phage-displayed library clones selected with SER4 were probed by ELISA to verify that the recovery of SER4-bound peptides had taken place during selection. All SER4-selected clones contained an identical sequence: INNEYVESPLYM. This mimotope sequence is predicted to be homologous to ^{87}AHNQVRQVPLQR^{98} in the ErbB-2 extracellular domain by means of clustalw search. The calculated diversity of a 12-mer peptide (4×10^{15}) greatly exceeded the actual diversity of the phage library (10^{13}) used for selection. This may be one reason for the rather low homology between the mimotope and the ErbB-2 homologous region. Since SER4 shows cell growth inhibitory activity, the predicted epitope region may be part of the functional domain of ErbB-2. Phage display–based epitope mapping of anti–ErbB-2 mAbs has been reported. In one report, a random peptide library displayed on phage was used (*40*), while a defined or random antigen fragment library displayed on phage was used in another (*41*). Although neither found a consensus sequence between the mimotope and ErbB-2, they successfully induced anti-tumor immunity by mimotope immunization. They concluded that tumor-suppressive ErbB-2 mAbs recognize conformational and discontinuous epitopes. Determination of the possible induction of anti-tumor immunity with the predicted epitope peptides is now in progress to clarify the nature and function of the SER4-defined epitope in ErbB-2.

The nucleotide sequence data reported in this paper appear in the DDBJ/EMBL/GenBank nucleotide sequence databases with accession numbers AB056117–AB056118. This work was supported by a grant from The Research Foundation For Pharmaceutical Sciences. We thank Dr. Dennis R. Burton (The Scripps Research Institute, USA) for the generous gift of pComb3 vector.

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