Construction, Structural Modeling of a Novel ScFv against Human Gastric Cancer from Phage-display Library

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Abstract

Using gastric cancer phage display library, a novel anti-human gastric cancer scFv, named as SC1 was screened. The SC1 gene was constructed and expressed in E. Coli. After sequencing, the heavy and light chain variable region of SC1 (named as SC1VH and SC1VL, respectively) was modeled using computer-guided modeling method. With molecular docking method, the 3-D complex structure of SC1VH and SC1VL, i.e. Fv fragment of SC1, was constructed and optimized with molecular dynamics method. Choosing the common linker (GGGGS) 3, the 3-D structure of SC1 was constructed and analyzed. The determination and analysis of the primary and 3-D structures of SC1 highlights the further human gastric cancer diagnosis and therapy.

Keywords: Gastric cancer, Computer homology modeling techniques, Single-chain antibody


1. Introduction

The function of antibodies is to recognize a theoretically unlimited set of foreign substances and to defend the vertebrate body against them. Because of their extraordinary affinity and specificity toward these antigens, antibodies are invaluable tools both in therapy and in research.

However, in spite of the monoclonal antibodies have many applications both in research and in the diagnosis and treatment of diseases, the usefulness of rodent monoclonal antibodies in human therapy is limited because of problems arising from their nonhuman origin (e.g. immunogenicity, variable efficiency in fixing complement [1, 2]).

With the development of phage-display library, large collections of antibodies with different specificity can be generated and selected based on the specific antigen-binding activity [3]. In the present research work, using human gastric cancer phage-display library, a novel anti-human gastric cancer antibody, named as SC1 was obtained.

Structural studies of antibody fragments have provided a basis for establishing the uniqueness of the (Ig) fold and the specificity of antigen recognition [4, 5]. The Ig fold is characterized by the β-barrel topology of each domain, combined with a high degree of conservation in specific packing residues, β-strand turns, and overall inter-domain packing geometry [6, 7]. Given the highly conserved structural motif, antibody fragment structures based on computer-guided molecular modeling method from the amino acid sequence have become possible [8, 9]. In the research work, after SC1 antibody heavy and light chain variable region (i.e. SC1VH and SC1VL) sequencing, the 3-D modeling structures of SC1VH and SC1VL were modeling using computer-guided homology modeling method, and the Fv fragment of SC1 was obtained using molecular docking method.

However, the variable domains of Fv fragments are not associated by covalent bonds, and consequently the domains tend to dissociate at low protein concentrations. One of the approaches to improve the stability of the Fv...
Fig. 1A Determination of CDR and FR frames of SC1V<sub>L</sub>

![CDR and FR frames of SC1V<sub>L</sub>](image)

Fig. 1B Determination of CDR and FR frames of SC1V<sub>H</sub>

![CDR and FR frames of SC1V<sub>H</sub>](image)
domain associate involves construction of single-chain Abs (scFv) in which the two variable domains are linked via a short flexible peptide. The most widely used linker designs have sequences consisting primarily of stretches of glycine and serine residues. As common, the linker (GGGGS)₃ was used to construct the scFv. In the present work, The SC1 gene was constructed and expressed in E. Coli. After sequencing, the heavy and light chain variable region of SC1 (named as SC1V_H and SC1V_L, respectively) was modeled using computer-guided modeling method. With molecular docking method, the 3-D complex structure of SC1V_H and SC1V_L, i.e. Fv fragment of SC1, was constructed and optimized with molecular dynamics method. using the common linker peptide, we formed the scFv_SC1. The studies are very important to the understanding of the specificity of anti-human gastric cancer antibody and may also shed light on the putative antibody recognition motifs for human gastric cancer.

2. Materials and Methods

2.1 Construction, selection and identification of the phage display library of anti-human gastric cancer single chain Fv antibody

Using the common protocol [10], a phage-display antibody library against human gastric cancer was produced, selected and identified. After sequencing the antibody SC1 variable region, each of the H- and L-chain sequences of the Fv fragment of SC1 (named SC1V_H and SC1V_L, respectively) is divided into seven segments, four framework (FR) regions and three CDRs according to Kabat’s rule.

2.2 Computer-guided homology modeling

Programs Homology was used to generate the 3-D modeling structure of the antibody SC1 Fv fragment. Using the BLASTP program deposited in http://www.ncbi.nlm.nih.gov/, the amino-acid sequences of the SC1V_H and SC1V_L were compared with the primary sequences of all immunoglobulins deposited in the Protein Data Bank.

The best match for the SC1V_H was the VH of the murine monoclonal antibody Ab2, where there was 85% identity of amino acid residues. The most homologous VL was the murine IgA 19.1.2 Fv, with 76.4% identity of amino acid residues. Using computer-guided homology modeling method, the 3-D theoretical structures of SC1V_H and SC1V_L was constructed.

2.3 Molecular dynamics refinement

The optimization of the initial models of SC1V_H and SC1V_L was carried out by the use of molecular mechanics and molecular dynamics techniques. The Discover and Discover-3 programs were used to generate the low-energy conformations of the CDR loops. An approximate solvent effect was introduced using the distance-dependent dielectric term in the electrostatic potential term. Harmonic constraints of 50 kCal/mol Å² were applied to fix the entire framework region of the model. Distance restraints were then applied to the atoms involved in the canonical conformations for the effective modeling of the loop regions. The system was subjected to 100ps of molecular dynamics at 300 K, and configurations were obtained through quenching of trajectories at 5-ps intervals.
2.4 Molecular Docking

According to the 3-D modeling structures of SC1VH and SC1VL, docking was performed to construct 3-D structure of the antibody SC1 Fv fragment using DOCKING module in Insight II 2005 software. A docking grid with a resolution of 8 Å including coulomb and van der Waals terms was created. The 3-D structure of SC1 Fv fragment was analyzed with regard to energy terms, intramolecular and inter-molecular interactions, and stereochemical property. The final structure of SC1 Fv fragment was submitted to a molecular dynamics calculation as mentioned above.

3. Results and Discussion

3.1 Expression, production, purification and characterization of SC1

The antibody SC1 expression unit in ABB expression vector was transformed into the *E. coli* JM 101 strain.

3.2 1-D primary structures of SC1VH and SC1VL

After DNA sequencing of SC1VH and SC1VL, the corresponding amino acid residues sequences of SC1VH and SC1VL were obtained with http://www.expasy.ch and shown in Fig.1. According to Kabat’s rule, the FRs and CDRs in SC1VH and SC1VL were also determined and shown in Fig.1.

3.3 3-D modeling structures of SC1VH and SC1VL

The amino acid sequence alignment between SC1VH and its best homologous protein was obtained with BLASTP program and shown in Fig.2A. Correspondingly, the amino acid sequence alignment of SC1VL was shown in Fig.2B. Based on the sequence alignment, using Homology modeling method, the original 3-D structures of SC1VH and SC1VL were predicted. And then, the two original structures were optimized and the final 3-D theoretical structures were shown in Fig.2A and 2B.

As shown in Fig.3, the net result of the CDRs in SC1VH and SC1VL possessed similar gross topologies, being somewhat convex with an apex at their first hypervariable region.

3.4 3-D optimized complex structure of SC1 Fv fragment

Based on the 3-D optimized structures of SC1VH and SC1VL, the 3-D complex structure of SC1 Fv fragment was obtained using molecular docking. With MM and MD optimization, the optimized 3-D complex structure of SC1 Fv fragment was constructed and shown in Fig.3.

The result in Fig.3 was shown that the binding sites between SC1VH and SC1VL formed a groove, running parallel to the VL:VH interface with depressions on the sides and a ‘pocket’ in the center, between the opposing L-CDR1 and H-CDR1 domains.

3.5 3-D theoretical structure of scFv-SC1

Using homology modeling method, the 3-D structure of scFv-SC1 was constructed and shown in Fig.4. Comparing with SC1 Fv fragment, the scFv-SC1 remains the active pocket as its Fv fragment, the stability was stronger than Fv fragment. The results was shown that the affinity and association to the target antigen of scFv-SC1 would be stronger than SC1 Fv fragment.

4. Conclusion

Knowledge-based approaches to antibody modeling are very useful to understand the relationship between structure and function of antibody. In the present work,
we obtained a novel anti-human gastric cancer antibody SC1 screened from phage-display library. The 3-D structures of the SC1VH, SC1VL, and SC1 Fv fragment were constructed and analyzed. The determination and analysis of the primary and 3-D structures of SC1 highlights the further human gastric cancer diagnosis and therapy.

References

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