·Informatics Research·

Bioinformatics-led design of single-chain antibody molecules targeting DNA sequences for retinoblastoma

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Abstract

• The development of single-chain Fv antibody (scFv) by recombinant gene expression is an important milestone for cancer therapy. Single-chain antibodies are reconstructed for cancer-targeted therapy to provide good penetration into tumor tissue and to improve their pharmacokinetics *in vivo*, offering a clinically valuable application. The relationship needs to be analyzed that there may be some variations between the structure and function of the fusion proteins, and the relationship between the structure and function of protein molecules was obtained through analyzing relevant literature at home and abroad as well as modeling analysis. Through our analysis of the interaction region between antibody and antigen, and of the binding sites for molecular conformation, it is clear that existing antibodies need to be modified at the DNA sequence level, enhancing the biological activity of the antibodies. Based on the view that bio-molecular computer models are closely integrated with biological experiments, a bio-molecular structure-activity relationship model can be established in terms of molecular conformation, physical and chemical properties and the biological activity of single-chain antibodies. Two enlightenments are obtained from our analysis. On one hand, the structure-activity relationship is clear for new immune molecules at the gene expression level. On the other hand, a single-chain antibody molecule can be designed and optimized for the cancer-oriented treatment. In this article, we provided the theoretical and experimental basis for the development of single-chain antibodies appropriate for retinoblastoma therapy.

• KEYWORDS: single-chain antibody; bioinformatics; retinoblastoma; structure-activity relationship

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INTRODUCTION

Retinoblastoma (RB)[1] is the most common malignant tumor involving the eye in the childhood, and its incidence is gradually increasing. This disease destroys severely the vision, eyeballs as well as lives of the patients. Current treatment modalities are just like that of comprehensive therapy, which includes nucleation, radiotherapy, chemotherapy, photodynamic therapy and thermotherapy. Of all, chemotherapeutic drugs can highly increase survival rates in the mid or late stage cases with micro metastasis. With their increasingly clinical application, the toxic effects and drug resistance of anti-cancer drugs also become severer.

A single-chain Fv antibody (scFv) is a protein comprising immunoglobulin heavy- and light-chain variable regions which are directly or indirectly connected [2]. In recent years, there has been some progress in cancer diagnosis and treatment using scFvs as carriers. However, the affinity and anti-tumor activity of a scFv can vary compared with that of the individual units. A designed inter-peptide linker is used to exploit the activity of scFvs, and this study concluded that the use of a suitable linker could allow a scFv to display full biological activity in clinical applications. The development of proteomics has led to an increase of studies on the composition, structure, and function of proteins. In studies

on relationships between the structure and activity of fusion proteins, bioinformatics provides a powerful tool. One important aspect of bioinformatics is drug design based on structural simulation of biological molecules. The simulation of spatial structures and molecular drug design, including studies on fusion proteins with various functional domains and inter-peptide linkers, is a current research focus[3-5].

MATERIALS AND METHODS

Materials In our experimental section, there exists the problem of unsatisfactory retinoblastoma scFv activity. This article expects to improve the experimental design further *via* analyzing relevant literature in recent years, thereby obtaining scFv with better activity. After 20 years of continuous in-depth study, scFvs have become increasingly valued in medicine, biology, immunology and many other disciplines. In particular, scFvs can maintain the affinity and specificity of the parental antibodies, allowing the fusion protein to bind to targeted cancer cells for diagnosis [6-8] or treatment of tumors [9-11]. Due to progress in theoretical research and clinical applications, scFvs have proven their utility. In particular, breakthroughs have been made in the fields of transformation of humanized antibodies and fusion proteins with linked immunotoxins: many humanized scFv antibodies have been approved by the U.S. FDA. In the laboratory, scFv research has made significant progress; however, there are still some problems for clinical applications, such as low affinity, poor stability and short half-life. These problems need to be resolved.

Methods The analysis of methods is divided into two stages: before 21st century and after 21st century. The first stage is defined as traditional stage, in which scientists mainly conducted experiments from the perspective of biological means. After entering 21st century, with the development of bioinformatics, people provide a transcendental method through bioinformation techniques, which allows to understand the relationship between the structure and function of derived factors of fusion protein before the experiment, thus to more scientifically and reasonably construct the fusion protein.

Traditional Analysis For effective preservation of antigen-antibody binding sites [12-14], it is essential that a single-chain antibody, comprising linked variable regions of heavy chain and light chains, folds correctly. In fact, many studies have shown [15-19] that the structure and function of fusion proteins might be different compared with those of the wild-type components. Fusion proteins can have a mutative dual activity compared with the wild-type function of each component, as assayed by detection of anti-tumor activity, possibly due to changes in the molecular conformation of the fusion proteins. The biological function of a protein derives from the characteristics of the native conformation or structure of the molecules. For fusion proteins to retain the activities of the linked units, the correct, native conformations must be formed. In the construction of single-chain antibodies, the affinity and stability of the fusion proteins within the spatial structure of the fusion protein determines whether the designed molecule has further penetrating power and a reasonable half-life. In recent years, there have been many studies on the design of single-chain antibody molecules[20-22]; however, it is a serious challenge for protein engineering to control the distance and orientation of the units of fusion proteins so that the function of the proteins can be optimized. Thus, it is important to understand how the structure of a protein relates to its function and mechanism of action. With the development of bioinformatics, computer-aided analysis systems have become essential tools for protein engineering.

Present Analysis At present, homology modeling is widely accepted as a reliable model for predicting the practical structure of proteins from their amino acid sequence. Homology modeling can reliably obtain the three-dimensional structure of a protein for analyzing the relation between its structure and function, and to identify active sites[23]. From the perspective of bioinformatics, homology modeling was adopted [24] to build the three-dimensional structure of a single-chain antibody for hepatocellular carcinoma. The three-dimensional structural information helped us to design a humanized single-chain antibody specific for hepatocellular carcinoma, and to determine the characteristics of the molecule on the epitope of the hepatoma cell.

Using bioinformatics, scientists have predicted and analyzed the spatial structure and the physical and chemical properties of antibodies, allowing the simulation of antigen binding sites, and even the design of new types of antibody molecules, to obtain high-affinity and low-antigenicity antibodies for enhancement of clinical applications. The spatial structures are mostly analyzed for the antibody bound to the antigen, but it is also very important, or even critical, that the hydrophobic interactions and electrostatic attractions are taken into account [25]. Thus, when antigen binding sites cannot be accurately predicted, the changes to the hydrophobic interactions and electrostatic attractions, within the context of the spatial structure, should be determined to aid in the identification of antigen-antibody binding sites. In practice, using computer-aided analysis systems [26], the hydrophobicity, isoelectric point, antigenicity, other physical and chemical properties, and the three-dimensional structure are predicted for an improved scFv. The computer-aided analysis can also provide information for studying the

Table 1 Analysis of the degree of influence of various factors on the spatial structure, physical and chemical properties and biological activity of an scFv

Factors	scFv				
	Spatial structure	Solubility and stability	Biological activity	Degradation	Biological expression
Length of the linker	+/-	+	+	+/-	+/-
Composition of the linker	+/-	+	+	+	+/-
Glycosylation of the sites	+/-	+	+	+/-	+
Hydrophobic interaction	+	+/-	+	+/-	+/-
Electrostatic attraction	+	+/-	+	+/-	+/-

(+: Influential; +/-: Controversial)

mechanism of the antigen-antibody reaction, as well as valuable information on the transformation of antigens and antibodies. However, the stability and immune activity of a scFv can be weakened, not only by the impact of the spatial structure and the physical and chemical properties between the VH and VL, but also by the lack of a quantitative relationship.

RESULTS

Inter –peptide Linker Designed for the scFv The composition, length and location of the linker between the heavy chain and light chain variable regions have impacts on the spatial structure, physical and chemical properties, and the biological activity of the fusion protein. Studies [27-29] have shown that it is very important that the flexible and hydrophobic peptide linkers join the functional domains of the protein without disturbing their functions. The use of different linkers will not change the isoelectric point, but the secondary structure of the proteins might be subtly altered. The most important function of a linker is to support and optimize the correct folding of molecules of the fusion proteins [30]. Unfortunately, there are no reliable selection criteria for designing linkers. Most linkers have been designed and selected by intuition, though there has been significant progress in the prediction of the secondary structure from the primary sequences of proteins; however, there is still very limited understanding of the relationship between the sequence and structure of proteins, especially of fusion proteins involving linkers. Intuitive selection of linkers often leads to uncertainty in the final conformation or activity of the fusion proteins, especially for longer linkers.

By experimental analysis, Shibata *et al* [31] suggested that linkers should be of sufficient length and good flexibility, to ensure that each unit of the fusion protein has enough freedom of movement and space to display their functions. On the basis of a large number of molecular biological experiments, scholars have reached the consensus that either linker can be too short to allow correct folding of protein antibodies due to intramolecular steric influence; or the linker can be too long to ameliorate the immunogenicity of the antibodies [32-34]. Computer-aided molecular design [35] and three-dimensional molecular modeling [36] are used with selected linkers for constructing molecular models of the spatial structure of single-chain antibodies; the spatial structure with the bound natural epitope is analyzed, which provides a further theoretical basis for the transformation of a single-chain antibody.

The length and sequence of the linker peptide can also significantly affect the expression and stability of a scFv[37,38]. The length of a linker is generally considered to be in the range of 6-25 amino acids when used to build bivalent single-chain antibodies. A certain degree of flexibility in the linker is required for the functional cooperation of the two subunits. Thus, successful construction of a scFv depends on the selection of a linker that neither interferes with the folding and association of the VH and VL domains, nor reduces the stability and recognition abilities of the Fv molecule. To satisfy these requirements, several design strategies have been developed. One approach is to use flexible glycine-rich sequences $(G_4S)n$ as tethers [39,40]. Other useful linkers are derived from multidomain proteins or selected by phage display [41]. However, as the complexity of the linker has a significant effect on the biological activity of the fusion proteins [42], the selection of the optimal composition and length of the linker for the desired activity of the fusion protein presents a challenge.

Impact of Other Factors on scFvs The composition, structure and nature of each component of fusion proteins should be taken into account when selecting linkers for different molecules. Studies have shown factors that affect the biological activity of the fusion protein include hydrophobic interactions and electrostatic attraction of fusion proteins [43-46] and the complexity of the linkers. The expression of a fusion protein with an inter-peptide linker can encounter problems not only related to solubility, stability and the fusion tag [47-49], but also related to serious degradation of the fusion protein. Many studies indicate that

the length of the inter-peptide linker, its amino acid composition, the existence of glycosylatio [50,51], the adaptability of the various units with the inter-peptide linker, and even the codons of the inter-peptide linker, can affect the function and stability of the fusion protein. However, quantitative models have not been established for explaining the relationships between the spatial structures, physical and chemical properties and the biological activity. Thus, the designed fusion proteins depend mainly on the experience of a "trial" and a "comparative" approach [52], and their optimization becomes problematic.

There are no reasons to believe, however, that a linker suitable for one antibody will be suitable for others, as can be deduced from Table 1. Indeed, the expression level, solubility, and stability of a scFv depend largely on linker length and sequence, and vary significantly from one linker to another [38,53]. At present, theoretical research on inter-peptide linkers is still limited for fusion proteins. Although the biological activity and expression of a fusion protein can be improved by optimization of the protein units, we hope to use bioinformatics to analyze and predict the amino acid sequence, secondary structure, and physical and chemical properties of fusion proteins to gain a better understanding of their biological functions. This will allow the production of stable fusion proteins at lower costs in a faster time.

DISCUSSION

Homology modeling has been successfully applied to the interpretation of the correlation between protein sequences, structures and functions. In particular, orthologous proteins often have conserved residues, and these residues can be interpreted by the protein 3D modeling. Using the structure model, multiple sequences of orthologous proteins can be compared and interpreted according to the restrictions of natural selection, requirements of protein folding, stability, dynamics and function. In effect, homology modeling can help us determine which functional groups the protein belongs to based on the analyses of critical conserved residues in the active sites and binding sites. Thus, homology modeling plays an important role in computer-aided drug design[12,54].

The entry point for the construction of single-chain antibody fusion proteins is the length of the inter-peptide linker and its effect on fusion protein activity. Linkers comprising repeats of G_4S [55] were used to construct bivalent single-chain antibodies targeting colorectal cancer with 5 and 15 amino acid linkers. With the 5 amino acid linker, the immune activity was unsatisfactory, possibly because the linker was too short to provide an effective distance for the two antigen-binding sites, and affected the stability of the cross-linked protein. On the other hand, the 15 amino acid linker was more conducive to correct folding of the antibody and ensured that the bivalent single-chain antibody retained its affinity and capacity. Gu *et al* [56] recently pointed out that the impact of linker length on the activity and affinity of engineered antibody depends strongly on the distance between N- and C-terminal of the V_H domain. This is probably why an antibody with a shorter linker peptide appeared to be a better candidate for targeting the CD20 antigen compared with its analog with a longer linker peptide. Thus, the dual approach that coordinates in-house molecular modeling and linker peptide design of an engineered antibody with quantitative determinations of antibody activity and affinity is required to optimize the construction of functional engineered antibodies. This study provided not only the rationale for designing novel engineered antibodies using molecular modeling, but also provided new insight into quantifying the binding affinity of the antibodies, especially at a low protein levels. The computer-aided homology modeling method used in the study [13] was demonstrated to be feasible and the modeling results were reliable. Molecular modeling could be used to guide scFv construction to obtain more satisfactory results. Thus bioinformatics is helping us to understand the relationship between structure and function of single-chain antibody fusion proteins.

In conclusion, studies have highlighted the relationship between an antibody's binding activity and its 3D conformation [56], which will provide direction for future antibody design and construction of IgM antibodies. Of course, further studies should be done to test the computer-aided modeling method, and the optimization of fusion proteins is still at the early stages of development. The factors that need to be considered for the comparison of the structure and function of two proteins include the End-to-end distance, the binding sites and the properties of the linker peptide. Other factors affecting the activity of scFvs will be discovered in future experiments; however, we hope that this article facilitates further research on single-chain antibodies. Using the model of molecular quantization, different correlation models can be established to simulate spatial structures for molecular drug design. Based on the databases of the natural protein structures and functions, the spatial structures and functions of the fusion proteins can be predicted using homology modeling. Combined with bioinformatics and genetic research, this can be used in searching and exploring new agents for gene therapy to combat retinoblastoma.

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