Genetic Engineering Antibody: Principles and Application

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Abstract. Antibodies are proteins secreted by plasma cells of the immune system to bind antigens and trigger the immune effector activity. Genetic engineering antibodies are novel recombinant antibody molecules with improved antigen specificities and effector functions, which produced by the recombinant DNA and protein engineering technologies. The ability to produce antibodies that are directed against specific antigens drives scientific discovery and the development of clinical diagnosis and therapy. This paper provides an overview of the current studies on the principle, categories, generation and application of genetic engineering antibody.

1. Introduction
Antibodies were first discovered at the end of 19th century. In the long process of antibody research, three generations of antibody technology have been developed successively. Polyclonal antibodies (pAbs) are made by different B cell lineages within the higher vertebrates, whereas monoclonal antibodies (mAbs) are secreted by a single cell lineage. Kohler and Mlstein first established hybridoma cells by cell hybridization in vitro and acquired a high purity monoclonal antibody for a specific antigen in 1975 [1]. By culturing the hybridoma cells in vitro, a large number of monoclonal antibodies are obtained. The second-generation technology avoids the dependence of higher vertebrates and greatly increases the production of antibodies. Because of the high specificity of monoclonal antibodies, it has been widely used in cell biology, basic medicine, clinical diagnosis and other fields [2]. The third generation of antibodies, genetically engineered antibodies, is made by the application of modern recombinant DNA or gene mutation technology to reconstruct gene fragments and obtain specific antibodies through cell transfection and culture in vitro [3].

2. Small-molecular antibody
The basic unit of a complete antibody contains two heavy chains and two light chains held together via disulfide bonds (Figure 1). Each polypeptide chain has a constant region (C) and a variable region (V). The fragment crystallizable (Fc) region contains only constant regions from the heavy chains (CH), but the fragment antigen-binding region (Fab) consists of heavy chain CH1-VH and complete light chains. The fragment variable region (Fv) contains only the two variable domains [4].

Because of the advantages of small molecular weight, such as excellent cell penetration, low antigenicity, efficiently expression in prokaryotic system and many other advantages, small molecular antibody has gradually become the research hotspot of genetic engineering antibody family. Major monovalent small molecular antibodies include Fab fragment, single-chain fragment variable fragment.
Polyvalent small molecule antibodies include double chain antibodies (Diabody), triple chain antibodies (Triabody) and Bispecific antibody (BsAb). The special type of small molecule antibody has the nanobody [5].

2.1. Fab

Fab fragment is an important functional area to ensure the immune affinity between antibody and antigen. Due to the absence of Fc fragment, small molecular antibody Fab has small molecular weight excellent cell penetration, low antigenicity compared with complete antibody, and still has the physiological activity of antigen-specific binding [6]. The Fab antibody library, constructed by phage antibody library technology, has been used in clinical areas, such as disease diagnosis, tumor therapy and the treatment of infectious diseases [7].

2.2. scFv

Fv fragment is the smallest unit of immunoglobulin molecular with antigen-binding activities [8]. scFv consists of variable regions of heavy (VH) and light chains (Figure 1), which are joined together by a flexible peptide linker that can be expressed in various expression system such as phage, yeast, plant, insect and mammalian cell [9]. The bacterial expression system is often applied for the production of scFv antibody compare to the various expression strategies available. The length and amino acid composition of the linker play an important role in the design of a viable linker peptide. The linker must make the heavy and light chain variable area freely folded in order to avoid affecting the antigen binding site and maintain the affinity [10]. scFv has been shown to be one of the best candidates for medical, diagnostic and research applications due to smaller size and less possibility of heterogeneous response.

2.3. BsAb

Bispecific antibody has drawn considerable attention due to their unique structure against two different antigens. Initially, BsAb was constructed though the chemical synthesis of two purified monoclonal antibodies, or though the fusion of two hybridoma cells. With the successful cloning of antibody genes and the rapid development of molecular biology technology, genetic engineering method that has better stability is used to design and produce recombinant BsAb with certain compositions. BsAb can be divided into two main categories according to structure, including bispecific antibodies with and without Fc fragments (IgG-like BsAb and non-IgG-like BsAb) [11]. IgG-like BsAbs have two Fab fragments and one Fc fragment, including Triomabs, DVD-IgG, KIH-IgG, CrossMab and so on. Unlike traditional monoclonal antibodies, two Fab fragments of BsAb are able to bind different antigens. Non-IgG-like BsAb consists of the VH and VL regions or Fab fragment from two different antibodies, including BiTE, DART, TandAb, bi-nanobody and so on. At present, BsAb is widely used in the field of tumor therapy. The first BsAb drug blinatumomab, approved by the FDA at 2014, can combine CD3 T-cell activator and B-cell antigen CD19 simultaneously [12].

2.4. Nanobody

The cameld species have unusual heavy chain IgG antibodies, devoid of light-chains and containing a single antigen binding variable domain (VHH) [13]. This VHH domain is also referred to as a nanobody with approximate molecular weight of 12-15 kDa (Figure 1) and are considered the smallest naturally derived antigen-binding fragment [14]. As a single domain antibody, the molecular structure of nanobody is relatively stable because nanobody lacks a connecting peptide and forms a disulfide bond inside. In addition to the advantages of small size, high affinity, less immunological effects and specificity, nanobodies are highly stable to extremes of pH and temperatures and can bind to their target at high concentrations of chaotropic agents [15]. The unique intrinsic properties of nanobodies make them better options for medical and biotechnological applications.
3. Humanized antibody

The B lymphocytes hybridization technique provides support for producing a large number of highly specific monoclonal antibodies and contributes significantly to the development of diagnostic and therapeutic antibodies. However, monoclonal antibodies are applied to the human body, which can stimulate the body's immune system to produce immune response, resulting in immune rejection [16]. Monoclonal antibodies also have some disadvantages such as larger molecular weight, larger molecule volume, and weak affinity of antibodies and antigens. Advances in genetic engineering and molecular genetics have prompted researchers to try to decrease or remove ineffective structures of antibody, increase or retain specific biological activity structures in gene level. The implementation of these enhancements can decrease or remove the murine structure of the antibody to reduce their immune rejection in the human body [17]. At present, the technique of antibody humanization mainly include Chimeric antibody and Complementarity Determining Reign grafted antibody (CDR).

3.1. Chimeric antibody

Chimeric antibody is produced by DNA recombination technology. The recombinant gene encoding variable region (V) of mouse antibody and the constant region (C) of human antibody are introduced into host cells for expressing a chimeric antibody. Humanized chimeric antibody retain the fragment (region V) of murine antibodies that have the ability to specifically bind antigens, and the fragment (region C) of human antibodies reduce the immunogenicity of antibodies in human body and the production of human anti-mouse antibodies [18].

3.2. CDR

In order to further reduce the murine structure of antibodies and increase the proportion of humanized structure in antibodies, the researchers developed Complementarity Determining Reign grafted antibody. This technique replaces other areas of the antibody with humanized structures, only...
retaining the CDR portion of the variable region of the murine antibody. The CDR technology greatly increases the humanization ratio of antibody, reduces its immunogenicity in the human body [19]. However, due to changes in the protein conformation, it may result in a decrease in antigen binding ability.

4. Antibody library
In the post-genome era, various genetic engineering antibody libraries are constructed on the basis of the molecular display technology platform in order to adapt to various "omics" studies, especially the high-throughput technology requirements of proteomics research [20]. The most commonly used molecular display technology platforms include phage display, yeast display and ribosome display [21]. The antibody library technology simulates and simplifies the production process of specific antibodies. Through the continuous optimization of specific antibody selection strategy and the design of antibody engineering, the new functions that antibodies cannot perform in their natural state are endowed.

4.1. Phage display
Phage display technology uses reverse PCR to clone a full set of genes encoded the fragment variable region of antibody. The antibody gene fuses with the phage shell protein gene, which causes the antibody molecular fragment to appear on the phage surface. Specific monoclonal phage antibodies are screened by the antigen-antibody affinity [22]. Phage display technology is first used to construct antibody libraries. And it's the most reliable and efficient method to produce therapeutic monoclonal antibody.

4.2. Yeast display
Yeast display technology is often used to construct antibody library such as scFv library, Fab library and IgG library [23, 24]. Compare with phage display, the most prominent advantage of yeast display technology is that the protein can occur post-translational modification, which greatly expanding the diversity of protein types. The yeast cells labeled by fluorescein and other markers can be used for quantitatively and real-time screening. Antibodies that recognize phenanthrene or methylphenanthrene and pH sensitive binding proteins are successfully screened using yeast display technology [25, 26].

4.3. Ribosome display
Ribosome display is the first method for screening and selection of functional proteins performed completely in vitro. In ribosome display, a mRNA–ribosem -protein complex that is used for selection can establish the physical link between genotype and phenotype [27]. Ribosome display has been used for improvement of the affinity and the stability of protein ligands, and for enrichment for soluble, folded proteins, and for selection for enzymatic activity [28, 29]. Using ribosome display technology, the researchers screened scFv that specifically bind to citrinin, and antibodies that both bind to soluble TNF alpha and transmembrane TNF alpha [30, 31].

5. Conclusions
The evolution preparation techniques of genetic engineering antibody have promoted the development and application of various antibodies with excellent performance and multifunctional antibody fusion proteins, and it also has a broad application prospect in the field of protein purification. With the continuous development of molecular biology and immunology technology, genetic engineering antibody will be widely used in basic scientific research, disease prevention, diagnosis and treatment, which provides strong support for raising the level of scientific research and improving the quality of human life.
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