Journal of Biosciences and Medicines, 2023, 11, 127-135
https://www.scirp.org/journal/jbm
ISSN Online: 2327-509X
ISSN Print: 2327-5081

Construction of Multi-Specific Antibody by Genetic Engineering and Its Progress in Tumor Therapy

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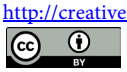
How to cite this paper: Xu, Z.Q., Gao, C., Jian, M.R. and Du, W. (2023) Construction of Multi-Specific Antibody by Genetic Engineering and Its Progress in Tumor Therapy. *Journal of Biosciences and Medicines*, **11**, 127-135.
https://doi.org/10.4236/jbm.2023.113013

Received: February 20, 2023
Accepted: March 21, 2023
Published: March 24, 2023



Abstract

Targeted treatment of cancer with monoclonal antibodies increases the benefit for patients. In order to improve the anti-tumor activity of monoclonal antibodies, multi-specific antibodies have entered the research field. The emergence of various techniques to produce multi-specific recombinant antibody molecules has led to the selection of target combinations in various forms. To date, only a few multi-specific constructs have entered phase III clinical trials, in contrast to classical monoclonal antibodies. Some of the format options are outlined from a technical point of view. We focus on the achievements and prospects of the underlying technologies for generating biand multispecific antibodies.

Keywords

Genetically Engineered, Multi-Specific Antibody, Tumor Therapy

1. Introduction

Tumor immunotherapy is a new direction of tumor treatment at present. Antibodies are secreted by plasma cells transformed from B lymphocytes, and each B lymphocyte strain can produce only one antibody specific to a specific antigenic determinant. With the development of cell and gene cloning technology, monoclonal antibodies have become the mainstream method for the treatment of cancer, but monoclonal antibodies only target one antigenic determinant, which is relatively simple and limited in treatment [1]. Multi-specific antibodies can recognize and bind two or more different antigens or epitopes at the same time,

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and block a variety of different signal pathways to play their role [2]. Compared with monoclonal antibodies, multi-specific antibodies can enhance tumor killing and increase binding specificity through the interaction of different cell surface antigens. The research and use of monoclonal antibodies make us further consider whether we can develop multi-specific antibodies to improve affinity and recognize multiple antigens on the surface of target cancer cells at the same time [3].

2. Multi-Specific Antibody

Multi-specific antibody is synthetic molecules that combine antigen-recognition sites from two antibodies into a single structure that can specifically bind multiple different antigens or epitopes simultaneously [4]. Multi-specific antibody has definite structure, composition, biochemical, functional and pharmacological properties, and have broad application potential in disease diagnosis, imaging, prevention and treatment [5]. Because of its specificity and versatility, it plays a more and more important role in tumor immunotherapy and brings hope for tumor patients [6]. The application of multi-specific antibody in tumor immunotherapy is as follows: 1) It can target antigens on tumor cells and receptors on T cells at the same time, so that T cells can connect with tumor cells, so that the immune system can better recognize and kill tumors. 2) Directly target tumor cells to play a role in killing tumors; 3) As the carrier of physical ability to accurately locate the therapeutic drugs or radioactive substances connected to it to the target area to reduce the damage of normal cells; 4) It can block multiple signaling pathways at the same time, kill tumor cells in a wide range and prevent drug resistance.

Multi-specific antibody is not a natural antibody, it needs to be prepared through a certain technology. Preparation of Multi-specific antibodies: 1) Chemical coupling method: This method was first used in 1985. The principle is that two complete monoclonal antibodies or Fab 2 fragments are coupled to form a bispecific antibody by chemical coupling agent. 2) Double hybrid tumor fusion method: Two kinds of hybrid tumor cells were synthesized by cell fusion method, and the stable target cell lines with two kinds of antibody functions were screened out. 3) Genetic engineering method: The use of genetic engineering technology to modify the antibody, so as to form a variety of forms of bispecific antibodies.

3. Common Immune Cell Recruitment Sites and Tumor Targeting Sites

3.1. Immune Cell Recruitment Site

Tlymphocytes originate from pluripotent stem cells in bone marrow and differentiate into mature T cells in the thymus. There are some leukocyte differentiation antigens expressed on T cell membrane, such as CD3, CD47, CD8 and so on [7]. CD molecules are the molecular basis of mutual recognition between T

lymphocytes and their subsets, which are widely involved in antigen recognition, cell adhesion and signal transduction of immune cells, and are the molecular basis of a series of important physiological and pathological processes such as inflammation, immune response, tumor metastasis and so on [8].

CD3 molecule is the most frequently used immune recruitment effector cell site in multi-specific antibodies. Among the multi-specific antibody structures, Catumaxomab targeting antigens developed by Trion Research/Neovii Biotech institutions are EpCAM/CD3 for the treatment of malignant ascites and Blinatumomab targeted antigens developed by Amgen institutions are used for acute lymphoblastic leukemia. Anti-CD3-anti-HER2-activated T cells developed by TransTarget/Barbara Ann Karmanos Cancer Institute is currently in phase II clinical study, and the targeted antigen HER2/CD3 Phase II can be used in breast cancer.

CD47 molecules are called integrin-related proteins. Inhibition of CD47 at immune checkpoints can effectively prevent tumor cells from escaping phagocytosis by macrophages [9]. At present, the study of multi-specific antibodies targeting CD47/CD19 and CD47/CD20 has achieved good results in killing tumor in vivo and in vitro.

The process of direct killing of tumor cells mediated by CD16 on human NK cells as lytic receptors depends on the close binding of antibodies. At present, the multi-specific antibody drug research related to CD16 is AFM-13 developed by Affimed Company, which is currently in phase II clinical study targeted CD30/CD16. The antibodies targeting CD19/CD16, EGFRVIII/CD16 and EGFRwt/CD16 are in the stage of research and development. These three antibodies are not only suitable for hematological tumors, but also can target some solid tumors [10].

3.2. Tumor Targeting Sites

Tumor targeting sites can be divided into tumor cell targeting sites and tumor vascular targeting sites. Tumor cell targeting uses specific antigens or receptors on the surface of tumor cells as targeting. Tumor vascular targeting works by using specific antigens or receptors on the surface of neovascularization capillary endothelial cells in the tumor vascular region. At present, the commonly used cell targeting sites are p53, EGFR, VEGF, CEA, CD20 and so on.

Epidermal Growth Factor Receptor (EGFR) is expressed on the surface of normal epithelial cells, but it is often over-expressed in some tumor cells. The over-expression of EGFR is related to the metastasis, invasion and poor prognosis of tumor cells. At present, the research of multi-specific antibody drugs related to EGFR includes Anti-CD3/anti-EGFR activated T cells targeting EGFR/CD3 of Barbara Ann Karmanos Cancer Institute mechanism for pleomorphic glioblastoma and pancreatic cancer. The Duligotuzumab targeted HEFR/HER3 of Genentech mechanism was used in the phase 1 clinical trial of solid tumors.

Vascular Endothelial Growth Factor (VEGF) is a highly specific vascular endothelial growth factor, which induces angiogenesis, promotes vascular permea-

bility, extra-cellular matrix degeneration, vascular endothelial cell migration, proliferation and angiogenesis in vivo. At present, the research of multi-specific antibody drugs related to VEGF has Vanucizumab targeting VEGFA/ANGPT2 of Roche mechanism for solid tumor and colorectal cancer in phase II clinical trial.

4. Construction Technique of Multi-Specific Antibody

The emergence of genetic engineering technology is the key driving force for the real development of multispecific antibodies. At present, multi-specific antibodies have been developed in many forms, among which the ones in tumor therapy can be divided into two categories: One is multi-specific antibody containing Fc segment, including trifunctional antibody (TrioMab), knobs-into-holesr structure antibody, CrossMab, IgG-scFv, Orth-Fab IgG, DVD-IgG, DAF bispecific antibody, etc. The other is BsAb, without Fc segment, including BiTE, DARTs, TandAbs and so on.

4.1. Construction of Multispecific Antibodies on DART and TRIEND Platforms

DART molecules can be expressed not only when there is a Fc domain, but also when there is no Fc domain, so it can be customized to have a longer or shorter half-life in vivo and it can induce or remove the effector function [11]. Connect two DART units or one DART unit and the Fab domain through the Fc domain (the latter structure is called the TRIEND structure). Mono-specific, bi-specific, tri-specific or tetra-specific molecules can produce up to tetravalent targeted antigens. Each peptide of DART molecule contains a VL domain, a VH domain, a cysteine residue that covalently connects two peptide chains and a coiled heterodimerization domain (**Figure 1(b)**). DART and TRIEND platforms are powerful toolkits for designing multi-specific antibodies, through which the valence of multi-specific antibodies can be regulated. Bivalent antibodies were designed, each specificity was univalent (1:1 format), each specificity was bivalent (2:2 format), one specificity was bivalent and the other was univalent (2:1 format), and each specificity was univalent trivalent antibody (1:1).

4.2. Construction of Multi-Specific Antibodies by BiTE Technique

BiTE (bispecific T cell engager) is a bispecific single-stranded antibody with T cells as effector cells. It has two antigen binding sites, which can bind T cells and antigenic molecules on the surface of tumor cells at the same time, so as to effectively activate resting T cells and kill tumor cells. BiTE technology uses DNA recombination technology to connect two different variable regions of light chain and heavy chain to a polypeptide chain (**Figure 1(a)**).

At present, BiTE antibodies have many targeted antigens, such as CD22, CEA, EGFR, CD19 and so on. The development of BiTE technology has greatly promoted the research and development of multi-specific antibodies and made a large number of multi-specific antibody drugs enter the clinical trial stage.

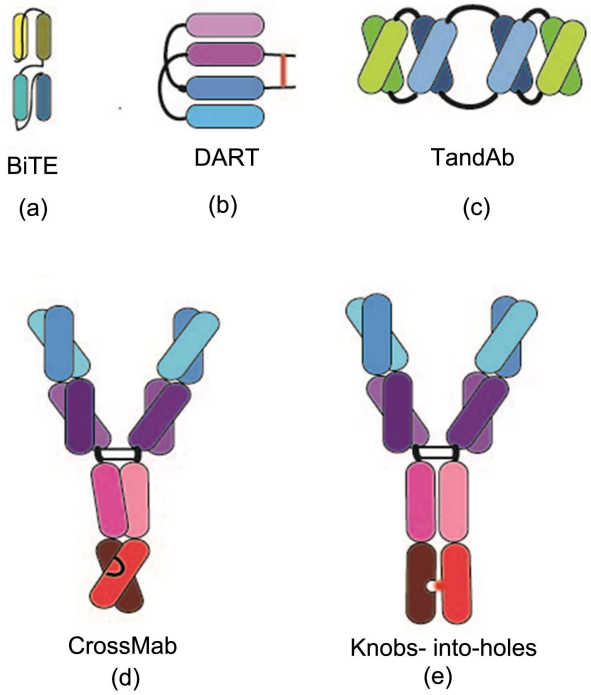

Figure 1. (a) Multi-specific antibodies constructed by BiTE techniques; (b) Multi-specific antibodies constructed by DART techniques; (c) Multi-specific antibodies constructed by TandAb techniques; (d) Multi-specific antibodies constructed by CrossMab techniques; (e) Multi-specific antibodies constructed by Knobs-into-holes techniques.

4.3. Construction of Multi-Specific Antibodies by Knobs-into-Holes Structure Antibody Technique

Knobs-into-holes (KIH) belong to the patent of Roche of the United States. This technique can assemble the heavy chains of two different antibodies into heterodimers. The multi-specific antibody was prepared by KIH technique. The smaller threonine (T) in the CH3 region of An antibody was replaced by a larger tryptophan (W) to form a "knob" structure (T366W), and three larger amino acids in the CH3 region of B antibody were mutated into smaller amino acids to form a "holes" structure (T366S L368A Y407V), which depended on the decrease of the steric hindrance effect after mutation and the covalent disulfide bond formed in the hinge region (**Figure 1(e)**). Promote heterologous heavy chain dimerization [12]. At present, a large number of multi-specific antibodies are prepared using this technique. The representative product of this technology is CEA-TEB, a new type of T cell multi-specific antibody, whose targeted CEA/CD3 is used to express carcinoembryonic antigen (CEA) in solid tumors [13].

4.4. Construction of Multi-Specific Antibodies by CrossMab Crossover Technique

CrossMab realizes the correct association of light chains by exchanging the domain of IgG to avoid some nonfunctional multi-specific antibodies due to light chain mismatch in the process of co-expression of the two antibodies in the same host cell. CrossMab was designed the KIH heterodimer connection in the

Fc part to achieve the complete exchange of light and heavy chain Fab part of (CrossMAb Fab), only variable region (CrossMAb VH-VL) or constant region (CrossMAb CH1-CL) exchange, in order to reduce light chain mismatch (**Figure 1(d)**) [14]. The representative product of this technique for the preparation of multi-specific antibodies is RG7221, which targets Ang-2/VEF, to block angiogenesis and is used in colorectal cancer.

4.5. Construction of Multi-Specific Antibody by Tandem Antibody (TandAbs) Technique

TandAb is two pairs of VL and VH domains connected by a single polypeptide chain. The short polypeptide chain makes it difficult to pair in the chain and forms TandAb. TandAb provides bivalent binding sites for each specificity, which increases the target binding affinity of each target antigen (**Figure 1(c)**). At present, the representative product of this technology for the preparation of multi-specific antibodies is AFM11, and its targeted CD19/CD3 is used in non-Hodgkin's lymphoma.

5. Summary and Prospect

At present, most of the targeted drugs used in the clinic are single targeted monoclonal antibodies, which have higher killing efficiency and lower side effects by killing tumor directly or indirectly. However, due to the multi-factors and complexity of tumor occurrence and development, it is difficult to obtain a good effect by relying on only one targeted molecule, and most monoclonal drugs have tolerance and recurrence in the process of use. Multi-targeted antibodies can target different target proteins at the same time, block the signal pathway that promotes tumor proliferation and immune escape, and further specifically kill tumor cells. With the help of DNA recombination and protein engineering technology, the immunoglobulin molecules were cut, spliced or modified at the

Table 1. Research progress of multi-specific antibody drugs.

Molecular Name	Target point	Molecular model	Indication	Development stage
Catumaxomab [15]	EPCAM + CD3	TrioMab	Malignant ascites	Approved
MT110 [16]	EPCAM + CD3	BiTE	Colorectal cancer	Phase I
Blinatumomab [17]	CD19 + CD3	BiTE	Acute myeloid leukemia	Approved
MT111 [18]	CEA + CD3	BiTE	Gastric adenocarcinoma	Phase I
MGD006 [19]	CD123 + CD3	DART	Acute myeloid leukemia	Phase I
MGD007 [20]	GPA33 + CD3	DART	colorectal cancer	Phase I
AFM11 [21]	CD3 + CD19	TandAb	Non-Hodgkin's lymphoma	Phase I
AFM13 [22]	CD30 + CD16	TandAb	Hodgkin's lymphoma	Phase I
RG7221	Ang-2 + VEF	CrossMab	colorectal cancer	Phase II
RG7802 [23]	CEA + CD3	CrossMab	Solid tumor	Phase I

gene level according to artificial design, and then reassembled into a new type of multi-specific antibody molecule. The recombinant protein retains the specificity and main biological activity of natural antibodies, and endows new molecules with new properties through modification, such as relative molecular weight, titer, flexibility, half-life and biological distribution. BiTE technology, DART technology, TandAb technology, CrossMab technology, Knobs-into-holes technology and so on are important methods for the research and design of multi-specific antibody drugs.

Although the improvement of technology has reduced the cost of preparing multi-specific antibody, there are still many problems in the design and construction of multi-specific antibody, such as improving the specificity, sensitivity and stability, increasing the half-life and selecting the appropriate target antigen to limit the external toxicity of tumors, etc. Therefore, the technology for preparing antibodies needs to be further optimized. Currently, multi-specific antibody Blinatumomab (also known as MGD011, which is a CD19 × CD3 DART) has been used in the treatment of B-cell hematological malignancies and has shown good anti-tumor effects [24]. Most bispecific antibodies for the treatment of solid tumors are in clinical research (**Table 1**). In the future, the advantages of multi-specific antibody drugs in tumor will be more prominent, and more multi-specific antibody drugs will be approved for tumor therapy.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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