Proceedings of ICBioMed 2024 Workshop: Computational Proteomics in Drug Discovery and Development from Medicinal Plants
DOI: 10.54254/2753-8818/75/2024.LA19333

The novel strategy of Bispecific Antibody-Drug Conjugates (BsADCs) in Cancer Therapy

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Abstract. Although conventional chemotherapy plays an important role in cancer therapy, its lack of specificity often leads to serious side effects and tolerability problems in patients. With obvious advantages in the field of tumor treatment, antibody-drug conjugates (ADCs) have gradually become a crucial research topic in recent years. However, some problems of current ADCs are being revealed, including the need to improve the internalization efficiency, the problem of drug resistance caused by tumor heterogeneity, and the reduction of targeting specificity. Therefore, bispecific antibody drug conjugates (BsADCs) become one of the directions for the development of next-generation ADCs. BsADCs can improve the endocytosis efficiency, the binding ability to tumor cells, and the killing ability of the drug through the bispecific epitope or targets while reducing the toxic side effects on normal cells, which has a promising prospect in the field of future development. This review collects some design concepts and innovative strategies in the field of current BsADCs, hoping to provide ideas and methods for further exploration and development of BsADCs and overcome the challenges it faces.

Keywords: Bispecific Antibody-Drug Conjugates, cancer therapy, targeting specificity, drug resistance.

1. Introduction

As one of the foremost global health challenges, cancer is characterized by the uncontrolled proliferation and aggressive growth of cells capable of invading adjacent tissues and metastasizing to distant sites. Traditional treatment for cancer, including surgery, radiotherapy, and chemotherapy, can inhibit tumor growth and spread to a certain extent, but the lack of specificity often causes damage to normal cells and brings major side effects [1]. Hence, the advent of targeted therapy led to creative advances in cancer treatment. This therapeutic approach aims to target the unique molecular markers of tumor cells, such as specific receptors, enzymes, or signal transduction pathways, to design and develop drugs that can specifically block tumor growth and metastasis. Compared with traditional chemotherapy, targeted therapy is more precise, more selective, less toxic, and has fewer side effects, so as to improve the therapeutic effect [2].

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Antibody-drug conjugates (ADCs) are an important way of cancer therapy, combining monoclonal antibodies with cytotoxic agents for targeted delivery. The antibodies bind to specific antigens on the surface of tumor cells, and the ADCs are endocytosed into tumor cells. Then the ADCs are degraded by lysosomes, and the cytotoxin is released to play an anti-tumor effect [3]. At present, there are 15 types of ADCs being approved for marketing in the world, which shows the advantages of strong targeting, high efficiency, and safety in the field of tumor therapy. However, with the in-depth application of ADCs in clinical practice, their inherent limitations are gradually exposed: intratumor and intertumor heterogeneity leads to drug resistance of ADCs, which limits the therapeutic efficacy of single antigen targeting; ADCs may express off-target toxicity in normal cells and tissues; the efficiency of cell internalization is low, and so forth [4].

Bispecific antibodies (BsAbs) are antibodies that can simultaneously recognize and bind two different antigens or two different epitopes on the same antigen. In this way, the specificity and affinity of the bispecific antibody binding to tumor cells are increased, and the damage to normal cells is reduced. In addition, it can also be designed to activate multiple signaling pathways in one cell type, producing synergistic effects and enhancing the immune response [5, 6]. Combining the dual-targeting ability of BsAbs with the potent cytotoxic effect of ADCs, Bispecific Antibody-Drug Conjugates (BsADCs) are developed with the advantages of different aspects. This design improves the ability to target multiple tumor populations within a heterogeneous tumor mass and engage immune cells for tumor killing in addition to direct cytotoxic effects. Dual-antigen targeting not only increases specificity and reduces off-target effects but also helps prevent resistance due to antigen escape or downregulation [7]. Therefore, BsADCs show great advantages in current cancer treatment and are regarded as a potential next-generation precision medicine. By analyzing the design strategies and key mechanisms of BsADCs, this review hopes to further explore the role of BsADC in enhancing cancer therapy.

2. Strategies for improving the efficiency of BsADCs

2.1. Target selection for BsADCs

Based on the current targeting of a single antigen and low internalization efficiency of some ADCs, we comprehensively analyzed some new treatment strategies and selected some new targets to construct dual-target ADCs to enhance the targeting and endocytosis of the drug. Studies have proven that EGFR (epidermal growth factor receptors) distributed on the surface of cell membranes of epithelial cells, fibroblasts, and other human tissues are expressed in a variety of solid tumors and have strong endocytic activity. However, embryonic trophoblast glycoprotein, which is rarely expressed in normal adult tissues but is expressed on the surface of embryonic tissues and various malignant tumor cells, including lung cancer, breast cancer, gastric cancer, etc., has been used as a target for the development of ADC drugs, but due to TPBG's weak endocytosis, it may cause certain difficulties in the development of ADC drugs targeting TPBG. The researchers constructed a bispecific antibody-drug conjugate by combining the TPBG antibody with the targeting antibody EGFR, which has high endocytic properties. By detecting the fluorescence signal intensity MFI value, they found that: by combining with EGFR to form BsADC, all three tumor cell lines, the endocytosis effect was enhanced to varying degrees, proving that the rapid endocytosis activity of EGFR drives and promotes TPBG endocytosis. In vitro killing experiments showed that BsADC significantly improved the killing effect on tumor cells. In the A431 model, the anti-tumor activity of BsADC was stronger than the synergistic effect of the respective parent monoclonal antibody ADCs [8].

Research reports have shown that in tumors that overexpress HER2, HER3 may be the core substance that enables HER2 to exert oncogenic activity [9]. The overexpression of both may lead to impaired survival in breast cancer patients. The researchers constructed three payloads conjugated to BsADC targeting HER2/HER3. The cell binding experiments of the antibodies showed that, compared with the control 2 V-MMAE and 3V-MMAE, the binding of 23 V-MMAE BsADC to JIMT-1 cells The ability is stronger. After BsAb is mixed with normal breast tissue cells MCF 10A, its binding rate to normal cells is very low, indicating that BsADC has an enhanced specific targeting effect on tumor cells [10].

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WTI is an antigenic factor that is minimally expressed in normal human tissues but is overexpressed in a variety of cancers, including serum and solid tumors. The researchers constructed a Bi-TCRm-ADC that inhibits both WT1 and NY-ESO-1 (a cancer-removing pill that was successful), which increases tumor suppression, reducing off-target effects [11].

2.2. Pegylated BsADCs

The problem of cytotoxicity is always a problem that ADCs hope to solve effectively. Macrophage FcγR recognizes and binds to the Fc moiety of the antibody, inducing the accumulation of IgG on the cell, and after binding to FcγR, ADCs with Fc fragments are endocytosed and transported to lysosomes for degradation [12]. For traditional ADCs, the small molecules released after degradation can kill immune cells, resulting in reduced activity and enhanced toxicity in vivo. Liu's team constructed a novel pegylated P-BsADC (JY207) targeting PD-L1 and CD47 and with PEG-MMAE (a pegylated cytotoxic payload MMAE). The drug is directly attached to the long PEG chain, and the resulting polyethanol-coupled drug is then attached to the dual antibody, making it free of Fc. The results showed that JY207b could still preferentially bind to CD47/PD-L1 double-positive tumor cells, thereby reducing the possibility of targeted toxicity, and was able to exhibit a strong killing effect, and JY207b showed a strong tumor inhibition at low doses in CDX and PDX models of transplanted tumor tissues from lung cancer patients [13]. The drug is currently in phase I clinical trials.

2.3. LITE-Technology in BsADCs

LITE technology is a method that utilizes small-molecule ligands to induce the transient binding of protein components, enabling precise control over the activity of biological drugs. By designing specific protein interfaces that respond to the binding of small molecule ligands, the formation and dissociation of protein complexes can be triggered. LITE technology demonstrates significant potential in biological drug development due to its unique advantages. Specifically, it enables precise temporal and spatial control over the formation and dissociation of protein complexes, thereby accurately modulating the activity of biological drugs. Furthermore, the presence or absence of small molecule ligands confers reversibility to the stability of protein complexes, acting as a dynamic switch for biological drug activity. Additionally, LITE technology exhibits flexibility by being applicable to a wide range of protein complexes, including antibodies, enzymes, and receptors, thereby offering diverse strategies for the design and application of biological drugs [14]. Bi-specific T cell cohesion (bsTCE): through LITE technology, can combine two antibody fragments for different antigens into a bi-specific antibody, the antibody in the presence of a specific small-molecule ligand to form an active complex to redirect T cells to kill tumor cells. Not only that, LITE technology in antibody-drug conjugates (ADC) also contributes. LITE technology can be used to control the binding and release in ADC to improve the accuracy and safety of drug delivery. In addition, precise regulation of the activity of immune checkpoint inhibitors can be achieved, thus avoiding immune-related adverse reactions [14].

To design and optimization of LITE technology, Structural and computational biology methods are employed to design protein interfaces with specific binding properties, ensuring efficient and selective triggering by small molecule ligands. High-throughput screening techniques are used to identify ligands from small molecule libraries that specifically bind to protein interfaces, optimizing binding affinity and pharmacokinetics. Adjustments to protein interfaces and small molecule ligand structures enhance complex stability under specific conditions, catering to diverse therapeutic needs. LITE technology faces challenges including the pharmacokinetics of small molecule ligands, the immunogenicity of protein complexes, and the complexity and cost of the technology itself. However, with ongoing technological advancements and optimizations, LITE technology promises to play an increasingly significant role in biological drug development, particularly in areas requiring precise drug activity control. LITE technology utilizes chemically induced dimerization (CID) domains to achieve switchable assembly of functional antibody complexes. Researchers employed LITE technology to transiently fuse IL-2 with an Fc domain, significantly extending IL-2's half-life in vivo from 8.6 minutes to 143.8 minutes while preserving its bioactivity through regulated dosing of small molecule activators like venetoclax. This

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technology holds potential to prolong the half-life of BsADCs and enhance their sustained therapeutic effects.

2.4. Non-Internalizing Design in BsADCs

The non-internalizing design is a crucial concept in the development of antibody-drug conjugates (ADCs) and other biological therapeutics. It focuses on minimizing or avoiding intracellular endocytosis triggered by antibody-antigen binding, influencing drug mechanisms and efficacy. By constructing antibodies or biological therapeutics that do not trigger cellular uptake mechanisms upon target binding, this design aims to exert therapeutic effects through distinct mechanisms, such as modulating cell surface signaling or inducing extracellular immune responses [15]. The core of non-internalizing ADC design lies in the linker between the antibody and the drug. These linkers are designed to cleave in the extracellular environment, releasing the payload without requiring cellular internalization or subsequent lysosomal degradation.

Non-internalized ADCs are able to effectively kill tumor cells without entering cells by acting directly on the cell surface, which may reduce drug resistance; meanwhile, non-internalized ADCs may be less toxic to normal cells due to reduced intracellular drug exposure, thus improving the safety of treatment. If not directly improving the internalization efficiency of BsADC, you can try to design non-internalized BsADC and choose some non-internalization or low internalization rate targets, such as targeting molecules in the tumor microenvironment (such as angiogenic factors, extracellular matrix components, etc.) that indirectly affect the growth and metastasis of tumor cells, or receptor activation or regulation of the immune system on immune cells, triggering an anti-tumor immune response. For example, BsADC, which can target vascular endothelial growth factor receptor (VE GF R) or programmed death ligand 1 (PD-L1) in the tumor microenvironment, can inhibit tumor growth by affecting angiogenesis or modulating the immune response independent of antigen internalization. This technique can also be combined with PEG technology to develop BsADC without Fc fragments, thus effectively reducing cytotoxicity [15].

The future development direction of non-internalized ADCs will focus on optimizing connector design to achieve higher stability and specificity fracture, screening efficient and low toxic drug load to enhance curative effect and reduce side effects, improving the antigen identification accuracy to enhance targeting, exploring the combined application strategy and immunotherapy to play synergistic effect, and according to the specific situation, so as to improve the effect and safety of non-internalized ADCs in tumor treatment [16].

2.5. Click Chemistry in BsADCs

Click chemistry, which was proposed in 2000, has been adopted as a new strategy for drug development, including the production of ADCs and synthesizing bispecific antibodies (bsAbs). Click chemistry has been awarded the 2022 Nobel Prize in Chemistry and uses a click reaction that binds a biomolecule to a reporter molecule. Its high yields, rapid reactions, and high reaction specificity have provided many chemical strategies in complex biological environments. Despite the numerous advantages of this new technology, its widespread use remains unapproved. However, there are many promising candidates in the clinical stage. Click chemistry may help increase the specificity of BsADCs by targeting tumor cells with overexpressed surface antigens. This also may increase the fast internalization and killing ability of BsADCs and contribute to the higher potency and efficacy of existing drugs [17].

In the past two decades or so, bioinorganic chemists and medicinal chemists have increasingly used click chemistry for the development of targeted drugs. The representative reaction of click chemistry is azide-alkyne cycloaddition (AAC), which has been used in ADC development. For example, the strain-promoted AAC (SPAAC) method, and some are currently being clinically evaluated for ADC applications [18, 19]. The transpeptidation and click reactions used in ADC production can be used for BsADCs. Anti-CD20/CD3 BsADCs are generated by copper-free sortase A-mediated click reactions and show T cell proliferation and activation [20]. The application of click chemistry in ADCs and

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BsADCs holds good prospects for the optimization of the combination of the two to form BsADC drugs and can promote better synergy between the two [21].

3. Summary

At present, 15 ADC drugs have been approved for marketing globally, but there is no bispecific ADC approved for marketing worldwide yet. Many products are in the stage of clinical Phase II and Phase III studies. The innovative strategy of BsADCs has opened up more development directions and fields for it, but there are still some problems: The current target selection of BsADCs is still somewhat limited, and it will also face problems such as low target expression efficiency and tumor heterogeneity. It is still necessary to carry out the development of other new targets. Although the targeting specificity can be enhanced at the antibody level, it is unable to fully improve the off-target toxicity of the drug. It is still necessary to conduct other new research in the fields of drug linkers and payloads to broaden the therapeutic window; The production process of BsADCs is more complex than that of traditional ADCs, and it may face problems such as peptide chain mispairing and complicated purification processes, so the optimization of the subsequent production part also needs to be considered.

Bispecific antibody drug conjugates represent an innovative direction in the field of cancer treatment and have broad development prospects. It shows great potential in tumor treatment. Bispecific antibodies can target two different antigens simultaneously, which makes them more effective at identifying and killing tumor cells. For example, it can simultaneously bind to antigens on the surface of tumor cells and CD3 molecules on the surface of T cells, thereby guiding T cells to attack tumor cells. And the design flexibility of bispecific antibodies enables them to target multiple tumor types and adapt to different treatment needs. This opens up the possibility of personalized treatment. However, it also faces some challenges. For example, due to production complexity, manufacturing bispecific antibodies is more complicated than monoclonal antibodies, resulting in high production costs and difficulty in ensuring batch-to-batch consistency. How to determine the optimal dose to balance efficacy and side effects is also a challenge. The high dose may lead to serious adverse reactions. Bispecific antibody-drug conjugates show promise in anti-tumor therapy, but overcoming current challenges requires interdisciplinary collaboration and innovation. Future research and development will likely provide patients with more effective and safe treatment options.

Authors Contribution

All the authors contributed equally and their names were listed in alphabetical order.

References

[1] Rasanpreet K , Alok B , Saurabh G .Cancer treatment therapies: traditional to modern approaches to combat cancers.[J].Molecular biology reports, 2023, 50(11):9663-9676.
[2] Sylva M R , Ahmed I S .Targeted therapies in cancer[J]. Surgery (Oxford), 2024, 42(3): 150-155.
[3] Lambert JM, Berkenblit A. Antibody-Drug Conjugates for Cancer Treatment. Annu Rev Med. 2018 Jan 29;69:191-207.
[4] Tsuchikama K, Anami Y, Ha SYY, Yamazaki CM. Exploring the next generation of antibody-drug conjugates. Nat Rev Clin Oncol. 2024 Mar;21(3):203-223.
[5] Li H, Er Saw P, Song E. Challenges and strategies for next-generation bispecific antibody-based antitumor therapeutics. Cell Mol Immunol. 2020 May;17(5):451-461.
[6] Blanco B, Domínguez-Alonso C, Alvarez-Vallina L. Bispecific Immunomodulatory Antibodies for Cancer Immunotherapy. Clin Cancer Res. 2021 Oct 15;27(20):5457-5464.
[7] Gu Y , Wang Z , Wang Y .Bispecific antibody drug conjugates: Making 1+1>2[J].Acta Pharmaceutica Sinica B, 2024, 14(5):1965-1986.
[8] Mei-qin JIN, Cheng-zhang SHANG, Yue-lei SHEN. Development and Anti-tumor Study of Bispecific Antibody Drug Conjugate Targeting TPBG and EGFR[J]. China Biotechnology, 2023, 43(5): 11-23.

DOI: 10.54254/2753-8818/75/2024.LA19333

[9] Tuen S L , Lisa C , Evelyn Y , et al.A central role for HER3 in HER2-amplified breast cancer: implications for targeted therapy.[J].Cancer research, 2008, 68(14):5878-87.

[10] Zong F` H , Li X , Han L , et al.A novel bispecific antibody drug conjugate targeting HER2 and HER3 with potent therapeutic efficacy against breast cancer.[J].Acta pharmacologica Sinica, 2024, 45(8):1727-1739.

[11] Shen Y , Li Y , Zhou J , et al.The Antitumor Activity of TCR-Mimic Antibody-Drug Conjugates (TCRm-ADCs) Targeting the Intracellular Wilms Tumor 1 (WT1) Oncoprotein[J]. International Journal of Molecular Sciences, 2019, 20(16):3912-3912.

[12] Manar H , W. A C , V. J L .Impact of Endocytosis Mechanisms for the Receptors Targeted by the Currently Approved Antibody-Drug Conjugates (ADCs)—A Necessity for Future ADC Research and Development[J]. Pharmaceuticals, 2021, 14(7):674-674.

[13] Shumin Liu, Weidong Lyu, Shuqiang Yin et al. Abstract 6307: A novel pegylated bispecific antibody-drug conjugate (P-BsADCpb-adc) targeting cancers co-expressing PD-L1 and CD47. Cancer Res 1 April 2023; 83 (7_Supplement): 6307.

[14] J A M , F E S , Suchitra P , et al.Switchable assembly and function of antibody complexes in vivo using a small molecule.[J].Proceedings of the National Academy of Sciences of the United States of America, 2022, 119(9):

[15] Ashman N, Bargh JD, Spring DR. Non-internalising antibody-drug conjugates. Chem Soc Rev. 2022 Nov 14;51(22):9182-9202.

[16] Subhan MA, Torchilin VP. Advances in Targeted Therapy of Breast Cancer with Antibody-Drug Conjugate. Pharmaceutics. 2023 Apr 14;15(4):1242.

[17] Hong, Y., Nam, S. -M., & Moon, A. (2023). Antibody–drug conjugates and bispecific antibodies targeting cancers: applications of click chemistry. Archives of Pharmacal Research, 46(1), 131–148.

[18] L C A , Xiaofan L , Millicent E , et al. Targeting CD74 in multiple myeloma with the novel, site-specific antibody-drug conjugate STRO-001.[J].Oncotarget, 2018, 9(102):37700-37714.

[19] Zammarchi F, Havenith KE, Chivers S, Hogg P, Bertelli F, Tyrer P, Janghra N, Reinert HW, Hartley JA, van Berkel PH. Preclinical Development of ADCT-601, a Novel Pyrrolobenzodiazepine Dimer-based Antibody-drug Conjugate Targeting AXL-expressing Cancers. Mol Cancer Ther. 2022 Apr 1;21(4):582-593.

[20] Wang Q , Chen Y , Park J , et al.Design and Production of Bispecific antibodies[J].Antibodies, 2019, 8(3):43

[21] Hong Y, Nam SM, Moon A. Antibody-drug conjugates and bispecific antibodies targeting cancers: applications of click chemistry. Arch Pharm Res. 2023 Mar;46(3):131-148.