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The application and development of antibody drug conjugates in cancer therapy

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Abstract. Antibody drug conjugates (ADC), as a further modification of monoclonal antibodies, deliver cytotoxic payload to targeted cells. It is a linker connecting a drug with an antibody. As an explosively expanding therapeutic field, ADCs have attracted huge attention among scientists and achieved significant progress during the recent decades. This is because antibody drug conjugates appeared to improve the specificity to target tumors while reducing the toxic side effects. Therefore, the advantages ADCs provided are appealing to both the academic and industrial field. Twelve ADC drugs have recently received FDA approval, while many more remain in the clinical development stage. This review article briefly summarizes the current applications of ADCs in cancer treatment, as well as the existing resistance mechanisms and potential future improvement to them in regard to its components. By providing more information in this field, this article is important in helping people understand antibody drug conjugates.

Keywords: Antibody Drug Conjugates, Target Cancer Treatment, Resistance.

1. Introduction

Chemotherapy, a therapeutic strategy that uses chemotherapy medications to destroy cancer cells, is one of the most widely used and dominant cancer regimens. Chemotherapy is a system-based treatment. Regardless of the route of administration (oral, intravenous, etc.), chemotherapeutic drugs will reach most of the body's organs and tissues along with the bloodstream. Therefore, at present, chemotherapy is the main or even the only treatment for certain tumours that tend to spread systematically and intermediate and advanced tumours that have metastasis. However, just because of this reason, chemotherapy caused a lot of side effects, there are many adverse reactions, such as hair loss, inhibition of bone marrow hematopoietic and so on. As a result, the strategy of cancer therapy is shifting toward precisely targeted therapies that target driver gene mutations. With the continuous development of targeted therapy, corresponding targeted therapy based on specific molecular phenotypes of tumors has become the first choice for advanced tumor therapy. Among them, monoclonal antibody drugs are famous targeted therapy strategies for specific targets. The monoclonal antibodies exhibit high

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susceptibility, high specificity, high efficacy and low toxicity properties. The clinical therapeutic effect is very outstanding, but there are still many bad effects.

To improve the targeting of cancer treatments and lessen harmful side effects, ADCs, a novel class of therapeutic medications, combine the high specificity of monoclonal antibody therapeutics with the high activity of small molecule cytotoxic agents. ADCs are cancer therapies made up of linkers, small chemical payloads, and antibodies that specifically target the hazardous payload of tumor cells. After entering the bloodstream and spreading to the tumor tissue, ADC attaches to tumor surface antigens, enters the tumor cells through endocytosis, and then travels to the lysosomes to release its payload. Toxic loads delivered through DNA damage or microtubule inhibition can lead to apoptosis, and they can also destroy nearby cancer cells due to bystander effects. Since the first ADC medication was introduced in 2000, there have been no drugs on the market for ten years, but nine drugs have been launched in the last ten years, indicating an upward trend when it comes to the ADCs' clinical development. Therefore, the development of ADC in the future is highly anticipated and will likely have positive clinical implications for future targeted therapies. This article focuses on the treatment of ADC drugs in different cancers, the current shortcomings of ADC, and the future improvement of this drug.

2. Application

As a promising type of immunotherapy, ADC has synchronous development of its efficacy and safety. The application of ADC for three kinds of tumors will be discussed in the following article.

2.1. HER2 breast cancer

ADCs hold great promise for treating patients with human epidermal growth factor receptor 2(HER2)-positive breast tumors, complying with the authorization and outstanding results of the drugs T-DM1 and T-Dxd [1]. Currently, 29 ADC candidates are currently being studied, all of which are at varying phases of clinical development [2]. Trastuzumab affects cell kill through multiple mechanisms, including inhibition of constitutive HER2 signaling and disruption of the HER2/HER3 interaction in HER2-overexpressing cells to inhibit cell proliferation." [1]. Furthermore, trastuzumab activates the immune effector system by binding to tumor cells and the FcyRIII receptor on immune effector cells, leading to antibody-dependent cell-mediated cytotoxicity [1]. Meanwhile, trastuzumab increased the antitumor activity of paclitaxel in breast cancer xenografts in vivo and it was also shown to enhance the toxicity of paclitaxel in HER2-overexpressing cancer cells in a dose-dependent manner in vitro. Trastuzumab was advanced for clinical evaluation based on those experimental data, and the FDA approved it for use in 1998 to treat patients with metastatic breast cancer whose tumors overexpress the HER2 protein [1].

2.2. Multiple myeloma

A B-cell cancer known as multiple myeloma causes unchecked plasma cell proliferation in the bone marrow. A diverse group of lymphomas, leukemias, and myelomas are known as B-cell lineage malignancies. Even though many new drugs are being developed to treat these cancers, many of them are still incurable with available treatments. ADCs of this class of drugs bind to cognate antigens on the surface of tumor cells (ADC-antigen complex through receptor-mediated endocytosis), release the cytotoxic payload to kill the target cells, and then internalize. To deliver cytotoxins to tumor cells preferentially, it capitalizes on of the inherent particularity of the interaction between antibodies and antigens. If the drug is not internalized, it may be released extracellularly and cause cell death [3]. There are a number of ADCs in development for myeloma, including anti-CD56 ADC, anti-CG74ADC, anti-CD38ADC, anti-CD269ADC, etc. [3].

2.3. Lymphoma

In recent years, ADC immunotherapy has taken its place in the treatment of lymphoma. Chemotherapeutic drugs, as an important pillar of hematological malignancies, have high potency but tumor selectivity, which may be related to non-specific toxicity and increased drug resistance. The

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therapy of lymphoma has been found to benefit from monoclones designed to specifically attach to cancer cell antigens. ADC has become a promising class of lymphoma immunotherapy [4].

Once bound to the appropriate cell surface antigens on cancer cells, ADCs are internalized and release cytotoxic payloads that lead to cell death, similar to the mechanism used to treat myeloma. However, regardless of antigen positivity, drugs can also spread to neighboring cells and cause cell death, which is called "bystander killing" [4]. Ideally, antigens would be uniformly expressed on malignant cells and almost absent on normal cells to maximize efficacy and minimize drug toxicity [4]. However, the efficiency of ADC use does not absolutely require the expression of "perfect" target antigens. Because heterogeneous tumors are likely to benefit from "bystander killing" that affects proximal antigen-negative tumor cells. Currently approved ADacs for lymphoma include Brentuximab vedotin (CD30), Polatuzumab vedotin (CD79b), and roncatuximab ticillin (CD19) [3].

3. Resistance mechanisms of ADC

Drug resistance has always been a problem in any drug field. Although antibody drug conjugates showed great effect on eliminating the tumor cells, they still have problems in long-term application. There are currently three main categories of resistance mechanisms to ADCs.

3.1. Antigen

ADCs are known for binding the antibody to the overexpressing antigens on tumor cell surface. However, not all the tumor cells have highly expressed target antigens. The situation might harden when downregulation or loss of the antigen occurs. For instance, the T-DM1-resistant cells have been observed to have a reduced expression of ErbB2 on the cell surface [5]. Another example is a breast cancer receptor. Reduced antigen expression of HER2 also help to explain the decreased efficacy of antibody drug conjugates. According to research, however, the expression of the HER2 antigen varies in person (from zero to overexpression), in which was caused by amplification of gene ERBB2 [6]. There are also examples about the complete deletion of an antigen. A report shows that the loss of CD33 on a person who had anaplastic large-cell lymphoma is the cause of the resistance to brentuximab vedotin [6].

3.2. Internalization trafficking pathway

After the engagement of antibody and antigen, the antibody drug conjugates are either taken in by endocytosis or pinocytosis. Then the cytotoxic payload must reach the nucleus to destroy the cancer cells. The pathway might be altered at this stage by changing lysosomal activity.

3.2.1. Impaired degradation in Lysosomes. In order to release payload, the ADC must be transported to lysosomes and go through degradation. However, payloads may not be released as successfully as expected. For example, the T-DM1-resistant cells take in antibody drug conjugates, but the intracellular caveolin then alter the trafficking of ADC to the lysosomes. Moreover, the function of lysosome can be affected by the level of pH. The cells which showed resistance to T-DM1 has a higher pH value in lysosomes, resulting in reduced lysosomal activity and ineffective proteolysis [7]. Besides, the lack of lysosomal transporters also results in insufficient ADC trafficking to the lysosomes. SLC46A3 expression loss is reported to be the reason for resistance to PBD and DM1 [8].

3.2.2. Cytoplasmic factors. The cytoplasmic elements presented in the cancer cells can also interrupt the engulfing process. To further explain, the substances in the intracellular fluid can lead to insufficient drug delivery to the lysosomes. For example, it has been shown that the polo-like kinase 1 (PLK1) proteins guard against the medication interference in the cell cycle [9].

3.3. Payload resistance

The process of the release of payloads into nucleus has been disrupted by several factors, involving the overexpression of medication efflux pumps and the deregulation of apoptotic mechanisms.

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3.3.1. Drug efflux pathway. The drug efflux pumps are to remove drugs including ADCs from intracellular cytoplasm. This mechanism is also known for causing resistance in chemotherapy field [10].

As a result of the fact that some of the ADCs are the substrates of the ATP-binding cassette transporters, the cytotoxic payloads end up being cleared from the cells. Consequently, the payloads are transferred out of the cells before producing an impact on the tumor cell genes. It has been noted that the T-DM1-resistant gastric cancer cell lines have elevated ABC transporters (ABCC2 & ABCG2). They are clearly associated with resistance as when inhibiting these transporters, the cells became sensitive to T-DM1 again [11]. Other experiments suggested that elimination of the MRP1 has the same effect. Further clinical data is needed to verify these statements, and there is a need for more research in the field.

3.3.2. Apoptotic pathway. Even if the payload is successfully released in the cytoplasmic fluid, the cells may not respond to it. This is due to the fact that antiapoptotic chemicals and substances are presented in the cell to prevent abnormal cell death. For instance, the naturally presented antiapoptotic proteins like BCL-2 and BCL-X have huge contribution on the resistance to a currently approved drug, gemtuzumab ozogamicin [10, 12].

4. Future potential

As the frontier research of the application of monoclonal antibody, ADC is a kind of anticancer drug with rapid development. Although there are still many shortcomings to be improved, it has strong development potential. The application of ADCs has improved with time, and given that they share a core of three components: antibodies that bind to cancer antigens, cytotoxic payloads, and connection-building linkers, the development and improvement of their efficacy is often based on these areas [12]. In addition, ADC can be improved in other aspects, including drug conjugation platform, antibody type selection, etc. [13].

4.1. Target Antigen

As with most antibody drugs, the selection and discovery of target antigens is a significant strategy for improvement. Regarding the choice of the optimal target, it is crucial to remember that the antigen is expressed at large levels on cancerous cells while being either hardly or not at all on non-cancerous cells. ADCs are intended to transmit cytotoxic payloads to all cells that express the relevant target antigen, so that malignant cells with higher priority expression have a wider therapeutic window than normal cells, thereby reducing systemic toxicity [12]. Currently, successful ADCs approved to treat solid tumors include HER2, TROP2, and nectin 4.

Among them, TROP-2, Trophoblast surface antigen 2, has emerged as a new cancer target in recent years. Initially, TROP2 was simply described as a trophoblast surface antigen, but recent studies have shown that it is expressed on some solid tumors, while its expression is not very strong in healthy cells. Sacituzumab govitecan is a clinically approved anti-trop2 ADC that is conjugated to the topoisomerase inhibitor SN-38, an irinotecan metabolite. The drug has shown encouraging results in clinical trials, especially in the clinical treatment of TNBC. Of the 108 TNBC patients included in the treatment analysis, 33 reported partial answers, with 33 reporting complete replies, with a median follow-up of 9.7 months, with a total response rate of 33.3% and a median duration of response of 7.7 months [14, 15]. After treatment, the ideal therapeutic effect was achieved.

In addition, the different basis of cell surface antigens and the internalization rate of antibodies also affect the efficacy of ADC. For example, the CD74 antigen quickly binds to the antibody and is internalized into the target cell. Preclinical data of immune-110 against CD74 suggest that it has similar effects to certain ADCs with slow internalization rate but strong payload toxicity [12]. Finally, in order to stop ADCs from degrading in the circulation and losing their effectiveness, it is also advisable to avoid choosing antigens that are strongly expressed in the circulation.

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4.2. Choice of Payload

Another area for improvement is the selection and development of payloads. Payload, commonly referred to as "cytotoxic compounds," is a crucial element influencing the functionality and characteristics of ADC. Currently, most commonly used payloads are derivatives based on natural products, including microtubule inhibitors (e.g., madenin derivatives), DNA damage agents (e.g., anthracyclines, pirrobenzodiazepine dimers), and DNA transcription inhibitors (e.g., primorids) [16].

The cytotoxicity of the payload almost determines the efficacy of the ADC drug and the adverse effects it produces. The number of antigens on each cell surface, the number of drug-payload molecules on each ADC, and the amount of time it takes for the antigen to return to the cell surface all affect how many intracellular payloads are present.

As a result, choosing the right payload is crucial for increasing effectiveness. Studies reveal that a very little portion of the dose given by tumor-targeted MAB medicines actually has an impact on the tumor tissue (about 0.1%), which means that ADC payloads need to be highly cytotoxic (IC50 at low nanomolar or picomolar levels) [17]. In addition, the payload also needs to have a clear target and mechanism of action, as well as chemical attachment points [16]. Therefore, the improvement of ADC payload is mainly carried out in these three aspects.

Recently, TR1801-ADC, a novel and highly effective antibody drug conjugated to cMet, has been found to be highly active in adult solid tumors. As a well-characterized oncogene, cMet is the target of many therapeutic agents, including antibody-drug conjugates. This drug, on the older generation of ADCs, is fully optimized, including specificity, stability, chemical sites, and so on. It has been shown that the antitumor activity of this drug is higher in different levels of cMet expression cell lines, while cMet-ADCs using tubulin inhibitors show a significant reduction in efficacy. Moreover, 90% of patients with head and neck, colorectal, and stomach cancers who received TR1801-ADC, from whom the model was generated, shown high link effectiveness [18].

4.3. Linker

The linker, which serves as a connector between the antibody and the payload, is the final crucial element. For the hazardous payload to be stably bound to the antibody, the linker must be stable in the plasma, but after recognition of the target, the drug enters the cancer cell or the lysosome, and the linker rapidly dissolves to allow the payload release to function. At present, there are mainly two kinds of linkers, which are non-cut type and cut type. Among them, the cleavable linker has a cleavage site, which can be cleaved by various mechanisms, such as reduction cleavage of disulfide bond and cutting with enzymes. However, the non-cleavable linker is composed of structures with anti-enzyme activity and is more stable in blood than the cleavable linker. Therefore, cleavable linker tends to have lower toxic side effects, stronger drug release efficiency and bystander effect than non-cleavable linker. Bystander effect means that the antigen-positive cells are targeted while also killing the surrounding cells [12].

As a result, the use of more stable linkers that are not susceptible to hydrolysis or other degradation mechanisms is one way to improve the ultimate efficacy. Current examples of improvements in linkers are the use of cleavable linkers to release drugs under certain conditions, such as low PH and enzyme activity. Just like the acid unstable linker, in the near-neutral environment of blood, the linker remains stable during the circulation process and accumulates in the target cells. After it is internalized, it enters the more acidic environment and leads to the decomposition of the linker [19].

Finally, under current clinical conditions, researchers are working to develop new linkers, such as photosensitive ADC linkers [19]. Besides, the use of site-specific coupling methods and the development of multifunctional linkers that can simultaneously target multiple biomarkers are also future directions.

5. Conclusion

ADCs are an emerging clinical treatment method on cancer patients. As a promising field that recently emerged, scientists have made great effort in discovery. In this review, we delve deep into the applications, downsides and prospects of ADCs.

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Through the deep research on the relevant articles, among the approved ADCs, we summarized the applications in breast cancers, myeloma and lymphoma. This newly invented type of drugs has played a significant role in all aforementioned fields, and more drugs are on their way.

Due to the fact that the ADCs do not exist until near decades, there are numerous limitations and problems related to their structure and components, waiting for solutions. One of the major concerns is drug resistance. During the procedure when ADCs come into effect, resistance mechanisms exist mainly dependent on the tumor cell marking, engulfing and cleavage process. Because ADCs work through releasing the cytotoxic payloads successfully into the correct target cell line, the downregulation of target antibodies and unexpected disrupting cytoplasmic factors, as well as the existence of normal cell protection pathway all contribute to the low efficacy of current ADCs.

In terms of the possible alterations that could improve the drug delivery of ADCs, specific methods relative to pathway and components of the ADCs are discussed in detail in this article. The target antigen should be selected carefully with consideration of expression levels both on tumor cells and in normal cell lines, preventing loss of effectiveness. Moreover, the crucial payload cytotoxicity and mechanism of action should also be taken into serious consideration as it directly determines the outcome. The linker stability and its optimum conditions are another aspect of future development. Further research efforts are needed to provide therapeutic and diagnostic evidence of these statements.

References

[1] Lambert, J. M. and Chari, R. V., "Ado-trastuzumab Emtansine (T-DM1): An antibody–drug conjugate (ADC) for HER2-positive breast cancer," Journal of Medicinal Chemistry 57(16), 6949–6964 (2014).

[2] Chu, Y., Zhou, X. and Wang, X., "Antibody-drug conjugates for the treatment of lymphoma: Clinical advances and latest progress," Journal of Hematology & Oncology 14(1) (2021).

[3] Herrera, A. F. and Molina, A., "Investigational antibody–drug conjugates for treatment of B-lineage malignancies," Clinical Lymphoma Myeloma and Leukemia 18(7) (2018).

[4] Chu, Y., Zhou, X. and Wang, X., "Antibody-drug conjugates for the treatment of lymphoma: Clinical advances and latest progress," Journal of Hematology & Oncology 14(1) (2021).

[5] Loganzo, F., Tan, X., Sung, M., Jin, G., Myers, J. S., et al, "Tumor cells chronically treated with a trastuzumab-maytansinoid antibody-drug conjugate develop varied resistance mechanisms but respond to alternate treatments," Molecular cancer therapeutics, 14(4), 952–963 (2015).

[6] Yardley, D. A., Kaufman, P. A., Huang, W., Krekow, L., Savin, M., et al, "Quantitative measurement of HER2 expression in breast cancers: comparison with 'real-world' routine HER2 testing in a multicenter Collaborative Biomarker Study and correlation with overall survival," Breast Cancer Research, 17(1), 41 (2015).

[7] Sung, M., Tan, X., Lu, B., Golas, J., Hosselet, C., et al, "Caveolae-Mediated Endocytosis as a Novel Mechanism of Resistance to Trastuzumab Emtansine (T-DM1)," Molecular Cancer Therapeutics, 17(1), 243–253 (2018).

[8] Kinneer, K., Meekin, J., Tiberghien, A. C., Tai, Y. T., Phipps, S., et al, "SLC46A3 as a Potential Predictive Biomarker for Antibody-Drug Conjugates Bearing Noncleavable Linked Maytansinoid and Pyrrolobenzodiazepine Warheads," Clinical Cancer Research, 24(24), 6570–6582 (2018).

[9] Hunter, F. W., Barker, H. R., Lipert, B., Rothé, F., Gebhart, G., et al, "Mechanisms of resistance to trastuzumab emtansine (T-DM1) in HER2-positive breast cancer," British Journal of Cancer, 122(5), 603–612 (2020).

[10] García-Alonso, S., Ocaña, A., and Pandiella, A, "Resistance to Antibody-Drug Conjugates," Cancer Research, 78(9), 2159–2165 (2018).

[11] Takegawa, N., Nonagase, Y., Yonesaka, K., Sakai, K., Maenishi, O, at el, "DS-8201a, a new HER2-targeting antibody-drug conjugate incorporating a novel DNA topoisomerase I inhibitor, overcomes HER2-positive gastric cancer T-DM1 resistance," International Journal of Cancer, 141(8), 1682–1689 (2017).

DOI: 10.54254/2753-8818/20/20230797

[12] Hafeez, U., Parakh, S., Gan, H.K., and Scott, A.M., "Antibody-Drug Conjugates for Cancer Therapy," Molecules (Basel, Switzerland) 25(20), 4764 (2020).
[13] Dean, A.Q., Luo, S., Twomey, J.D., and Zhang, B., "Targeting cancer with antibody-drug conjugates: Promises and challenges," mAbs 13(1), 1951427 (2021).
[14] Goldenberg, D.M., Stein, R., and Sharkey, R.M., "The emergence of trophoblast cell-surface antigen 2 (TROP-2) as a novel cancer target," Oncotarget 9(48), 28989–29006 (2018).
[15] Syed, Y.Y., "Sacituzumab Govitecan: First Approval," Drugs 80(10), 1019–1025 (2020).
[16] Kostova, V., Désos, P., Starck, J.-B., and Kotschy, A., "The Chemistry Behind ADCs," Pharmaceuticals (Basel, Switzerland) 14(5), 442 (2021).
[17] Drago, J.Z., Modi, S., and Chandarlapaty, S., "Unlocking the potential of antibody-drug conjugates for cancer therapy," Nature Reviews. Clinical Oncology 18(6), 327–344 (2021).
[18] Gymnopoulos, M., Betancourt, O., Blot, V., Fujita, R., Galvan, D., et al., "TR1801-ADC: a highly potent cMet antibody-drug conjugate with high activity in patient-derived xenograft models of solid tumors," Molecular Oncology 14(1), 54–68 (2020).
[19] Sheyi, R., de la Torre, B.G., and Albericio, F., "Linkers: An Assurance for Controlled Delivery of Antibody-Drug Conjugate," Pharmaceutics 14(2), 396 (2022).