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Mechanisms and Optimization Strategies for the Occurrence of Antibody-Drug Conjugate Toxicity

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Abstract: Antibody-drug conjugates (ADCs) have emerged as a promising class of targeted cancer therapies, with 13 ADCs approved by the FDA and over 140 undergoing clinical research. ADCs are designed to enhance the selective delivery of cytotoxic drugs to tumor cells through high-affinity monoclonal antibodies (mAbs), improving the therapeutic index of the drug. Despite their potential, ADCs face significant challenges related to toxicity, including hematologic, ocular, skin, and neurological side effects, which limit their clinical application. This review provides an in-depth analysis of the mechanisms underlying ADC toxicity, which includes both targeted and off-target effects that can damage normal cells and tissues. Strategies for optimizing ADC safety, such as improving antibody structure, effector molecules, linkers, and combination therapies, are discussed. The review emphasizes the need for continued research to address these challenges and reduce off-target toxicity while enhancing the efficacy of ADCs, paving the way for the development of safer and more effective next-generation ADC drugs.

Keywords: Antibody-drug conjugate, targeted therapy, toxicity, strategy of optimization

1. Introduction

Atibody-drug conjugate (ADC) has developed rapidly in recent decades. Currently, 13 ADCs have been approved by FDA, and more than 140 ADCs are under clinical research [1]. Because most ADCs use effector molecules that are less effective and significantly toxic when administered alone (i.e., "free drugs"), ADCs were originally developed to enhance selective delivery of drugs to cancer cells by targeting specific tumor cells and tissues with high-affinity monoclonal antibodies (mAbs). Thus, the therapeutic index of the drug is improved. However, ADCs also face the challenge of toxicity in clinical application. These marketed ADCs also have different degrees of toxicity, such as hematologic toxicity (thrombocytopenia, neutropenia), ocular toxicity, skin toxicity, peripheral neuropathy, etc. These side effects not only reduce the therapeutic effect, but also may cause serious adverse reactions and even death, which limits the clinical application of ADC.

Therefore, this review will discuss and optimize the design scheme of ADC from the microscopic molecular structure of ADC to the macroscopic mechanism of action in human body to reduce toxicity, ensure the safety and efficacy of ADC, and point out the research direction for the next generation of ADC drugs.

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2. Mechanisms of ADC toxicity

2.1. Targeted toxicity

Targeted toxicity refers to the killing effect of ADC on non-tumor tissues but normal tissues or cells with the same target antigen. Ideal targets for ADCs are those that are expressed only on tumor tissue or that have a huge difference in expression between tumor tissue and normal tissue. If there is no significant difference in the expression of target antigens between tumor cells and non-tumor tissues, the killing effect of ADC on tumor cells will inevitably damage normal tissues or cells with the same target antigens. For example, due to expression of nectin-4, the target of enfortu-mabvedotin, in the salivary glands, approximately 40% of patients treated with the drug develop dysgeusia, which is thought to be due to toxic side effects of the drug's Nectin-4 target antigen in the salivary glands [2]. In addition, risk markers of severe cardiotoxicity and decreased left ventricular ejection fraction were observed in patients treated with trastuzumab based ADC [3]. Targeting toxicity is not only determined by target antigen expression, but may also be related to the mechanism of action of the payload, the accessibility of the ADC to the target cells, and the regenerative potential of the target cells/target organs. trastuzumab emtansine (T-DM1) is the first ADC approved for patients with metastatic breast cancer that is positive for human epidermal growth factor receptor 2 (HER2), which is strongly expressed on cardiomyocytes as well as epithelial cells of the digestive tract, skin, lung, and breast. However, thrombocytopenia and elevated liver enzymes were observed in clinical trials to control the major dose-limiting toxicity of the drug [4]. In the non-clinical safety evaluation of targeted toxicity, the expression of target antigen in normal tissues and organs needs to be taken into account when designing animal experiments, and it needs to be evaluated in cross-reactive species. Of course, in the process of clinical evaluation and use of ADCs, targeting toxicity rarely becomes a major problem, and drugs that cause severe targeting toxicity have a high probability of being rapidly diagnosed in the early stage of preclinical development, thus screening ADCs that can enter clinical research.

2.2. Off-target toxicity

Off-target toxicity refers to the killing effect of ADC in tissues or cells that do not express the target antigen, which is mostly related to the safety of the payload of cytotoxic small molecule compounds and is highly similar to the common dose-limiting toxicity of ADCs in clinical practice. At present, the main mechanism of off-target toxicity is thought to be related to coupling stability and non-specific uptake by normal cells [5]

2.2.1. Stability of coupling

The type of linker and the chemical coupling technique are the main factors affecting the stability. The ideal linker should ensure the stability of the coupled compound in vivo and the ease of release at the target site, so as to ensure both safety and efficiency. The linker is divided into cleavable and non-cleavable. "Wherein cleavable linkers contain chemically or enzymatically susceptible chemicals designed to exploit specific conditions that are unique to the intracellular or tumor extracellular environment, with the goal of maintaining good stability of the systemic circulation and rapid cleavage of the target site." In practice, cleavable linkers are often hydrolyzed in plasma at an appreciable rate, leading to premature release of payloads in extra-tumor compartments. Non-cleavable linkers are more stable than cleavable linkers and require intracellular proteolysis and complete metabolism of ADCs to produce cytotoxic metabolites, most of which are composed of intact adaptor effector molecules attached to antibody-conjugated amino acid residues. Examples include lysine-SMCC-DM1 of trastuzumab emtansine or cysteine-MC-MMAF of belantamab

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mafodotin. However, it also has disadvantages. Its overly stable linker makes it difficult to play the role of ADC when the bystander effect is needed [5]. For example, POLSON et al. combined a series of antibodies against non-Hodgkin lymphoma antigens and effector molecules in non-targeted safety studies in rats [6], and found that DM1 could be connected with cleavable (SPP) or non-cleavable (SMCC) linker to construct a variety of ADCs, and proved that both efficacy and safety in vivo were good. SMCC-ADC showed anti-tumor effect against only 2 of 7 target antigens, while SPP-ADC was effective against all targets. However, at the dose of 20 mg/kg-1 ADC, mice treated with SPP-ADC showed more significant weight loss, liver toxicity and blood toxicity than mice treated with SMCC-ADC.

2.2.2. Cell-specific uptake

The non-specific uptake of ADC by normal cells is also an important reason for the off-target toxicity of ADC, but the current research on this mechanism needs to be further studied. Antibody can be taken up by normal cells in an antigen-independent manner through various mechanisms, such as nonspecific uptake by Kupffer cells and liver sinusoidal endothelial cells, which increases the occurrence of ADC drug-related liver injury. Alternatively, positively charged loading molecules interact with negatively charged cell membranes to promote nonspecific endocytic uptake [7]. The Fc domain of the antibody interacts with the Fc receptor (FcγR) expressed by immune cells, resulting in off-target uptake and toxicity of ADC. For example, T-DM1 activates the uptake of FcγR by macrophages. Results in frequent thrombocytopenia and the occurrence of interters-titial lung disease (ILD) [3]. These toxic mechanisms allow ADC to eliminate cancer cells while also causing certain damage to normal tissues, which limits its safety and clinical efficacy. In-depth exploration of these mechanisms is essential to further optimize ADC design to ensure more stable, safe and specific drug delivery effects, thereby minimizing toxic side effects. In this section, different optimization schemes for the structure of ADC small molecules are proposed, and a comprehensive and detailed scheme analysis is carried out.

3. ADC toxicity optimization method

3.1. Optimizing Antibodies

The traditional conjugation method is to randomly couple the effector molecule to the amino group of the antibody lysine or the sulfhydryl group exposed by the reduction of the interchain disulfide bond, but it has a high risk of toxicity. The latest method to reduce toxicity is bispecific antibodies targeting two tumor-associated antigens, because only cells with high expression of both antigens will bind to ADC. Thus enhancing selectivity and improving the ability of tumor cell endocytosis. The first clinical data were released in September 2022, with a HER2-specific ADC ZW49 (targeting two nonoverlapping HER2 epitopes) showing an objective response rate of 31% [8]. At present, other dual-specificity ADC developments have also been reported, including HER2/Trop2, HER2/CD63, HER2/integrin, HER2/PRLR, EGFR/c-Met, EGFR/MUC1 and EGFR/HER3 [9]. Attempts were also made to silence the Fc region of the antibody to reduce the FC-binding uptake of ADC induced by immune cells and to reduce the off-target and extra-tumor toxicity. In order to improve the therapeutic index of ADC, some studies have attempted to connect two different types of drug payloads to the same monoclonal antibody, such as combining HER2-targeting antibody with MMAE and MMAF, and FGF2-targeting antibody with MMAE and α-amanitin [8].

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3.2. Optimize the design of the linker molecules

The chemical linker is a key component of the ADC, which connects mAb and cytotoxic payload. The adaptor helps the ADC to maintain stability in circulation until the ADC reaches the target tumor cell and releases the payload. Since the unstable concentration of ADC in plasma can lead to increased off-target toxicity, the second-generation ADCs used non cleavable linkers to reduce the possibility of off-target. However, ADCs with cleavable linkers generally exhibit superior bystander effects, so the application of ADCs containing non cleavable linkers is limited and is currently mainly used to treat hematologic cancers or tumors with high antigen expression. The late introduction of cleavable valine-citrulline dipeptide linker (Val-Cit) is more stable in blood circulation, but it is susceptible to the influence of peptidases in the blood and produces off-target toxicity. The third-generation ADC was further optimized based on the combination of dipeptide linker with p-aminobinylcarbamate (PABA) and maleimide spacer (MC) to obtain better cathepsin B binding ability and better plasma stability. Another strategy is to develop tripeptide and tetrapeptide-linker agents with tissue specific peptidase activity (MC-GGFG-AM), which significantly reduce blood clearance and exhibit excellent circulatory stability [10].

Another milestone in the conjugation strategy of the third-generation ADCs is the use of site-directed conjugation technology, such as the latest use of AJICAP second generation, and the use of Fc affinity reagent to specifically modify the new coupling site (Lys288), and the conversion rate is high. This coupling technology can easily introduce two payload connectors for each natural antibody and produce more than 20 site-specific ADCs, which will not aggregate during the reaction process. It is compatible with various antibody forms and improves drug uniformity and stability, so as to achieve better tolerance and obtain predictable pharmacokinetic indicators [11]. In addition to site-directed binding, another engineering strategy is to change the length of the polyethylene glycol (PEG) group in the linker to increase the hydropathy/decrease the hydrophobicity of the ADC to reduce toxicity and improve efficacy [12]. In recent years, with the development of peptidyl linker technology, some researchers have developed an anti-CD79BADC with tandem cleavage linker, which contains β-glucuronic acid groups and shows obvious plasma stability and in vivo efficacy [13]. The use of self-cleaving chemical linker, such as para-aminobenzyloxycarbo-nyl (PABC), can significantly improve the stability, targeting and tolerance of ADC due to its rapid intracellular proteolysis. Among the 13 approved ADCs, this linker has been applied to 4 MMAE-ADCs, which has achieved remarkable results. In addition, ADCs are prone to antibody aggregation due to the higher DAR employed, but the antibody aggregation problem of offspring ADCs has been minimized by optimizing linker/loading, DAR, and conjugation chemistry [14].

3.3. Optimization of effector molecules

Effector molecules are the root cause of ADC toxicity. Therefore, structural modification based on effector molecules may also improve the safety of ADC. Traditionally, structural modification of effector molecules is to generate effector molecules with new structures in order to obtain effector molecules with reduced toxicity on the basis of maintaining activity. For example, MILLER et al. carried out structural modification of PBD dimer and produced a new DNA alkylating agent molecule, IGN [15], by reducing an imine group that reacted with DNA. Compared to ADCs with PBD dimers as effector molecules, ADCs with IGN as effector molecules exhibit stronger bystander effects due to the easier release of IGN from DNA adducts. This advantage in vitro translated into superior antitumor effect in vivo. In addition, mice tolerated 6 mg/kg of ADCs with IGN as the effector molecule, but only 2.8 mg/kg of ADCs with PBD dimer as the effector molecule.

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3.4. Toxicity was suppressed by combination therapy

Researchers have innovated a combination therapy consisting of CA4 and anti-PD-L1 drugs. They have developed a nanotechnology-based formulation of CA4 [16]. To develop poly (L-aspartic acid) -polyethylene glycol (PEG)/costatin A4 nanoparticles to improve the water solubility and prolong the half-life of CA4. The active drug is slowly released from the nanocavitary, ensuring a constant plasma concentration in the tumor, thereby reducing toxicity. Another approach is to use polymeric CA4 nanoparticles in combination with sorafenib [17], a well-established anticancer agent targeting VEGF-A. The combination of CA4-NPs (30 mg/kg) and sorafenib (30 mg/kg) resulted in over 90% tumor inhibition with minimal liver toxicity in the H22 subcutaneous tumor model.

4. Conclusion

In this review, the toxicity mechanism of ADC is introduced in detail, mainly due to the targeted toxicity and off-target toxicity of ADC, which may lead to damage to normal cells and tissues. Most ADCs still cause frequent and even life-threatening toxic effects. At present, many strategies for ADC toxicity are expected to improve the safety of ADC, including reasonable optimization of antibodies, effector molecules, linkers, and combination drugs. In the future, more comprehensive analysis and validation methods are still needed to ensure the stability and efficiency of ADC drugs. How to reduce the off-target toxicity of effector molecules and give full play to the advantages of ADC technology through new design concepts is still a key issue that needs to be paid attention to in the new generation of ADC design.

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