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Antibody-Drug Conjugate and Its Application on Lung Cancer

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Abstract:

Antibody-drug conjugates (ADCs) represent a promising class of anticancer therapeutics, comprising a monoclonal antibody (mAb) linked to a cytotoxin via a specialized linker. Currently, 15 ADCs have received FDA approval, with numerous others undergoing clinical trials. Despite their potential, ADCs have shown limited application primarily in specific cancer types such as adenocarcinoma, and their efficacy in other cancers like non-small cell lung cancer (NSCLC) remains under exploration. This paper reviews the history of ADC development and delves into the mechanisms underlying their function. It provides a detailed analysis of the various components of ADCs—antibodies, linkers, and cytotoxins—and how their selection impacts the overall efficacy and safety of the drug. Specific targets, such as HER2, and corresponding ADCs are also discussed. Additionally, the challenges associated with ADC application, including poor target specificity and the risk of severe off-target effects, are highlighted as critical areas for ongoing research and development.

Keywords: Antibody-drug conjugates; monoclonal antibody; non-small cell lung cancer.

1. Introduction

Cancer is one of the most malignant diseases on earth, with 20 million new cases and 9.7 million kills in the year 2022, becoming one of the greatest killers of humanity. Moreover, with 2.5 million new cases, lung cancer has become the top of all varieties of cancer, which is 12.5% of all cancer patients [1].

One of the most widely used therapies for cancer is chemotherapy, which is mostly about taking cytotoxin and kill the cancer cells. It does this by going into cells that are replicating and destroying their double helix structure, like alkylating agent, or forbidding the DNA that forms microtubules to divide, known as microtubule stabilizer [2]. The usage of it started by 1947 and is still commonly used, but it also has some drawbacks due to the target of replicating cells, which could also kill liver cells, bone marrows, hair, and so on.

The proposal of mAbs provides a more effective solution for immunotherapy. Due to the antibody's specificity, it can target the pathogen by targeting the antigens, and then triggering the immune system [3]. This mechanism can support the targeting of cytotoxins

In fact, by the year 1910, there was already a concept called "magic bullet" proposed by Paul Ehrlich,

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trying to produce a medicine that only kill the pathogen but not the normal cells. In the next few decades, a lot of efforts were put into this concept but only little harvest. Finally, during the 1960s, thanks to further understanding of antibodies, people figured the track of using antibodies as the way to achieve target, and the concept of antibody-drug conjugate (ADC) was first proposed, but it is only after another decade, Milstein and Köhler produced hybridoma and gain the ability to produce mAbs, giving ADC a practical way to produce the specific antibody they need. Therefore, after years of further development and testing, the first FDA-approved ADC finally appeared in 2000. Nowadays, there are more than 100 FDA approved ADC drugs, trying to cure cancer [4].

Though there are 15 kinds of ADCs, there's only one for the biggest cancer, lung cancer. There are millions of people in need of this kind of medicine, the society needs ADC for this specific cancer.

Based on the mechanism of ADC drugs, this paper will focus on the antibody, cytotoxin, and linkage ADC can use to cure non-small-cell lung cancer (NSCLC); antigens that are targeted by, especially HER2; current trials on ADC on NSCLC; and problems currently faced by ADC manufacturers.

2. Building Blocks of ADCs

The creation of ADC requires three parts: mAb, cytotoxin, and linker, and to combine them, a conjugation method is needed. For a mature ADC, it is expected to precisely target and release the toxin inside the target cell, which is NSCLC cells in this paper.

2.1 Antibody

By using antibody, an antigen to target on is needed. An ideal antigen in cancer cells should be either only present on NSCLC cell or overly expressed on it, which can make sure drugs will have a minor chance to harm normal cells. Also, the antigen has to be expressed at the surface of the cell, therefore it is more likely to be attached by ADCs [4-6]. In addition, being a non-secreted antigen is needed so that ADCs won't be attracted by some floating antigen [4]. Finally, the receptor should pull the ADC inside after they attach, which is called internalizing, therefore pull the cytotoxin inside and destroy cancer. There are few that does it, some examples that exist in NSCLC are HER2, HER3, Trop2 [7-9]. HER2, for instance is about 100 times more expressed on tumor cells than normal cells, which makes antibodies less likely to harm normal cells [4]

The recommended antibody for ADCs is immunoglobulin G, or IgG. It is the most common type of antibody in our body and have multiple traits that fits this job. First, it has a long half-life, which means after injecting it into human body, it will last longer than any other antibody [4]. There are 4 types of IgG, which are IgG1, IgG2, IgG3, and IgG4. IgG1 is the most commonly used type due to the presence of Fc receptor (FcRn) mechanism and therefore can last very long and trigger strong effector functions such as antibody-dependent cell-mediated cytotoxicity (ADCC) that natural killer (NK) cells have and complement-dependent cytotoxicity (CDC) [4].

2.2 Cytotoxin

Cytotoxin is the bullet ADC is carrying to kill the tumor cells after internalizing. There are also a few conditions needed as an appropriate cytotoxin. First, only about 2% of the ADC injected into the body can accomplish this task of internalizing [4,10], so making the cytotoxin more toxic and guarantee that it can kill the tumor cell is necessary. Plus, the cytotoxin is ideal to be stable and able to conjugate with the antibody [10]. There are generally two types of Cytotoxin: antimicrotubule agents like Auristatins or Maytansinoids that can stop the microtubules from working and stop cell division; The other one, DNA damaging agents like Exatecans, Calicheamicins, and so on, are able to destroy DNA structure therefore killing the cancer cell [4,6].

ADC usage in lung cancer is however being questioned due to its toxicity. The number first risk is the off-target effects due to early release of cytotoxins while circulating or binding to the correct antigen but the wrong cell, this can cause serious damage to the lungs [5]. Another risk is the bystander effect, which is when the cytotoxins escape from the cancer cell after being released in it and go to nearby cells, which could be both cancer or non-cancer cells, people usually allow it to happen since there's a higher chance that cells around cancer is still cancer, but lungs are relatively more vulnerable. The possibility of this happening would increase if the affinity of the target antibody. Finally, inflammation could happen if through ADCC, the immune system gets overly activated, and causes trouble in the body, ending up with interstitial lung disease, or ILD [5,11]. These risks in lung cancer are one of the main issues that obstruct the application of ADC in lung cancer and are one of the focuses of future studies.

2.3 Linker

The linker is a long chemical compound that connects the antibody and the cytotoxic. It is ideal to be stable and only release the drug after internalizing. There are two types of linkers, cleavable and non-cleavable [4,6].

Non-cleavable linkers mean the linker will theoretically not break in the body, therefore things like off-target

effects wouldn't happen, and drugs won't fall off and harm unlucky cells. The only way it can release drug is by breaking the antibody, after internalizing, the protease would break down the ADC but release the cytotoxins, and accomplish its goal. Though the ADC broke down, the drug is still connected to a piece of antibody [4,12], which can make sure it won't get out of the cell and cause bystander effect [13].

Next, cleavable linkers are the more commonly used type, it means that the linker can degrade and release the drug. Most of the times, the linkers get degraded in front of a certain pH, enzyme, or some chemical. First, pH ones can break at a certain pH, usually in an acidic environment since endosome(pH5~6) and lysosomes (pH4.8) [4,14], inside cells, are acidic. Therefore, after internalizing, ADC will reach the acidic environment and release the drugs. Second, it is the enzyme sensitive linkers, and the lysosomal protease is the enzyme needed. These proteases are generally overly expressed in cancer cells and protease inhibitors are extant in circulatory system, and can prevent drugs from being released before reaching the target [4]. The major chemical sensitive linker uses reductive glutathione (GSH) since it helps cell division, it is common in cancer cells.

2.4 Conjugation Method

Other than the materials, the way to assemble them is also crucial in ADC production. On the antibody, there are extra lysine and cysteine which allow connecting [4], and there are three ways to connect. The first way is to use cysteine's thiol group and connect to it, it can therefore create a cleavage linker. The second way is to use enzymes, mainly formyl glycine generating enzyme (FGE), transglutaminases, glycotransferases, and sortases. The last one is by using unnatural amino acids (UAAs), for example, the most commonly used ones are Azinomethyl-L-phenylalanine, Acetylphenylalanine, and Azide lysine [10].

3. Targets in Lung Cancer

3.1 HER3

HER3, or human epidermal growth factor receptor 3, a receptor located at the cell surface, is part of the HER, also can be called EGFR family; after these receptors get activated, the cell will replicate rapidly and start cell migration, if it happens too often, tumor will show up. About 60% of lung cancer patients overexpresses this protein [8], and giving it the quality of a preferable target. The only ADC drug that targets HER3 is Patritumab-Dxd, but none of them are approved by FDA [5].

3.2 Trop-2

Trop-2 is a glycoprotein located at the cell surface. It is overexpressed in multiple kinds of cancer cells including NSCLC. By regulating calcium ions, it is able to fasten cell division and migration therefore causing lung cancer. Since it is overly expressed, on the surface, and will do internalization, it fulfilled all the previous said requirement and is a preferred target for ADC.

The ongoing clinical trials on Trop-2 use drugs like Dato-DXd, sancituzumab govitecan [5], and SKB264 combined with EGFR-TKI, which is in phase 2 and targets for NSCLC. There's great potential in ADC that targets Trop-2.

3.3 HER2

HER2, or human epidermal growth factor receptor 2, is a common target used in lung cancer; in about 10%-30% of NSCLC, HER2 proteins are overly expressed [6], there will be 25-50 times more HER2 than other normal cells. HER2, like rest of the HER family, after activation, can cause cell division, cell migration, and form blood vessels around the cell, these are the traits that help with tumor forming and leading to cancer. Among the HER family, HER2 are more resilient to internalization and degradation, in other words, a better target [6].

HER-2 is one of the most wildly used target in the ADC that cures NSCLC. First, Ado-trastuzumab emtansine, or T-DM1 is the first ADC to be targeting HER2, the DM1, which is a microtubule agent, is connected to the antibody by a non-cleavable linker. In a phase II study on its efficacy on NSCLC, T-DM1 showed high efficacy when facing HER2 exon 20 mutation [6].

Next, it is the Trastuzumab deruxtecan, also known as T-DXd. It is made up of a IgG1 and a DXd drug connected with a cleavable linker, it inhibits the camptothecin topoisomerase and destroys the DNA. In the experiment, I showed potent activity, but also raised safety concerns, more trials need to be tested on to limit the harm on human body. Eventually, FDA approves its application in NSCLC at a dose of 5.4 mg/kg [6].

4. Problems to be Solved

4.1 Cytotoxin

The biggest concern hindering large doses is the risk that more ADC will kill the patient. Since the only way to let ADC enter the body is by injection, it has the risk of poisoning the blood, which is the main side effect of all 14

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FDA approved ADC drugs. If the drug accidentally falls before entering the target cell, the drug that is wandering around will randomly kill cells in the endocrine system, weakening the transportation and the immune system. Moreover, in the study of HER2 targeting ADC, ILD is a common side effect. The exact mechanism is unknown, but a common hypothesis is that it is due to the amount of blood coming and the long period of time they stay, causing a higher chance of cytotoxins falling into lung cells and causing ILD. [4]

4.2 Target Reaching

The rate of ADC actually entering cancer cells and killing it is poor, this is because the size of ADC is huge compared to typical ones: the antibody part is a huge protein. Therefore, only a small portion of ADC injected can reach the tumor cell. To solve that, people use cleavable linkers and cause the bystander effect, which did enhance the influence of the drug, but increases the risk of poisoning. [4]

4.3 Drug Resistance

One of the major challenges faced by medication is drug resistance, ADC is no exception. However, the mechanism is much more complex than typical ones and some parts of it haven't been proved correct. There is proof that cancer cells will reduce the production of the target receptor after exposing to a certain drug for a long time [4], or a lot of different mechanism that get away with ADC, for example, for T-DM1, a drug mentioned earlier, the cancer cells will reduce the expression of the receptor, constantly use a ligand to bind with the receptor so that internalization wouldn't happen, lower the lysosome's potency so that the drug can't release, or use channels to transport ADC out of the cell [15]. It is amazing how cancer cells can create such a complex mechanism to resist ADC and the creativity of cancer is frustrating dozens of doctors and patients. Also, the cancer can simply ignore the drug itself, just by slightly changing the sequence of certain protein, drugs might lose their effect and escape from elimination [15].

5. Conclusion

This article focuses on the main components of ADCs - antibodies, cytotoxins and linkers. Further analysis found that antibodies such as IgG, especially IgG1, are favored because of their long half-life and ability to induce a strong immune response. Cytotoxins, including anti-microtubule agents and DNA damaging agents, are essential to ensure that even a small portion of the ADC that reaches cancer cells can effectively induce cell death. In the selection of linkers, the choice between cleavable and non-cleavable linkers is also critical because it affects the stability of the ADC and its ability to release cytotoxins in target cells.

In addition, several key targets of ADCs in NSCLC, including HER2, HER3 and Trop-2, the current research status and related clinical trials are also further analyzed in this article. For example, T-DM1 and T-DXd have shown promise in clinical trials, but subsequent studies still need to focus on their toxicity and drug resistance improvements.

This complex and uprising field is now giving hope to thousands of patients, and a great deal of effort in the different fields for different parts of ADC is made to finally form this special kind of therapy, more and more drugs with similar mechanisms are emerging, like peptide drug conjugate, PDC, but this field still requires more development in the on-target rate and human body harm.

ADC has the potential to improve specificity and reduce side effects compared to traditional therapies. However, continued research is needed to address current limitations, including optimizing the composition of ADCs, overcoming drug resistance, and the development of safe and effective delivery systems.

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