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Research on immunotoxins and their applications

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Abstract:

This article discusses the research progress and application of immunotoxins in cancer therapy. Immunotoxins,are proteins that combine a toxin with an antibody or growth factor, specifically attacking cells with a particular marker. show a high degree of specificity and lethality against cancer cells. The paper reviews the history of immunotoxins, their classification, mechanism of action and their application in the treatment of different types of cancer, emphasizing their potential to reduce immunogenicity and prolong the treatment cycle. Despite challenges such as immunogenicity in clinical applications, immunotoxins are considered to be powerful tools for future cancer therapy.

Keywords: cancer therapy, specificity, toxin, antibody

1. Introduction

In recent years, cancer has become one of the leading causes of death in humans, with WHO statistics suggesting nearly 20.2 million deaths globally in the last 10 years (dedicated to the development/implementation of cancer treatment programs/follow-up healthcare spending)[1]. In 2020, the number of new cancer cases is expected to be about 20 million and the number of new deaths is expected to be about 10 million.[2] If left unchecked, the eventual burden of disease is likely to bring about the collapse of the healthcare system, and hence the urgency of cancer treatment. Currently cancer treatment methods include chemotherapy, radiotherapy and surgery. However, these meathods are generally characterized by non-specificity, i.e., while attempting to remove tumor cells, normally dividing and differentiating cells may also suffer some irreversible damage, thereby reducing the prognosis of survival rates and quality of life[3].

Immunotoxins, also known as biological missiles, are proteins that contain toxins and antibodies or growth factors that bind specifically to target cells, and are a class of fusion proteins specifically designed to selectively destroy cells bearing a particular marker. It is usually synthesized by chemical cross-linking of a highly specific monoclonal antibody and a powerful killing toxin molecule, which needs to bind to and internalize the target cell, and the enzymatic fragment of the toxin has to be transferred into the cytoplasm. Once the toxin molecule enters the cytoplasm, it kills the cell, and a single toxin molecule can kill a cell by inactivating EF2 or ribosomes every minute. In contrast, it takes thousands of toxic molecules to kill a cell [4].

Immunotoxin overcomes the shortcomings of traditional chemotherapy and radiotherapy methods for treating tumors. It has both the specific recognition function and the killing function of toxin. It is one of the advanced and effective cancer treatment methods by combining with the receptor antigen on the surface of tumor cells and then internalizing it, followed by inhibiting protein synthesis in the cell leading to the death of tumor cells without damaging the normal tissues.

2. Content

2.1 History of ITS development

First generation immunotoxins, made from full-length PE proteins attached to intact monoclonal antibodies. However, these immunotoxins can bind to normal cells. The defects of first generation immunotoxins are loss of specificity, low stability and heterogeneity of components. [5] used chemical conjugates of antibodies to intact toxins or toxins with attenuated cell binding properties. Although they have shown tumor regression in some lymphoma patients, they are usually ineffective because the constructs are heterogeneous, nonspecific, and too large to infiltrate solid tumors [6]

The second generation of Immunotoxins, removed areas of the toxin that were not Important for cell killing, and the resulting toxin fragments bound to specific antibodies and could not bind to normal cells. This led to the creation of a small, truncated toxin called PE38, which can attach to cells by attaching to antibodies, but cannot bind or kill cells when used alone. However, its large molecular size

limits its entry into tumors and some immunotoxins attach to normal cells, which can cause conditions such as vascular leakage syndrome (VLS) and hemolytic uremic syndrome, side effects that have been improved in the design of third-generation immunotoxins [5] The third-generation immunotoxins are designed to eliminate heterogeneity and reduce the immunogenicity and size of the Its through DNA recombination techniques. Smaller sized antibody fragments are used as targeting molecules, which in turn reduces immunogenicity and increases IT penetration in solid tumors. Immunotoxins containing antibody FV fragments are called recombinant immunotoxins [4]. They include single-chain variable fragments (scF) single-chain variable fragments (scFv), disulfide-stabilized variable fragments (dsFv) fragments (dsFv) and antigen-binding fragments (Fab) genetically fused to truncated and de-immunized toxins [7].

Fourth-generation immunotoxins, which are composed of humanized or humanized antibody fragments incorporating cytotoxic proteins of human origin, such as cytotoxic enzymes (proteases and Rnases) and cytostatic proteins, thereby minimizing off-target toxicity and immunogenicity.

2.2 Mechanisms of ITS in cancer therapy

The binding of the receptor-binding domain in the immunotoxin molecule to the corresponding antigen or receptor on the surface of the target cell allows the entry of the immunotoxin into the cell via receptor-mediated endocytosis, which facilitates the translocation of the toxin molecule into the cytoplasm through the processes of internalization and transmembrane translocation. This is followed by inhibition of protein synthesis through intracellular catalysis via ADP-ribosylation of dithioamide residues of elongation factor 2 (EF2), leading to cell death [6].

3. Classification of ITS

3.1 Classification according to the toxin molecule:

(1)bacterial toxin

For example: Pseudomonase exotoxin (PE), diphtheriatoxin (DT), anthrax toxin (anthraxoxin), Shigatoxin, cholera toxin (choleratoxin), etc.[8]

These protein toxins are used to bind to targeting molecules, such as antibodies or growth factors, with the aim of killing cells bearing specific antigens or receptors. Due to their targeted nature and small effective dose (just a few toxin molecules can kill a cell), these protein toxins are used to treat autoimmune diseases and transplant rejection. In particular, immunotoxins constructed against tumor-specific antigens (TSA) and tumor-associated antigens (TAA) on the surface of tumor cells have been widely used in targeted therapy of tumors [9].

(2)phytotoxin

For example: ricin, saporin, gelonin and pokeweed antiviral protein.

The phytotoxin consists of a catalytic A chain, which is bound to the cell surface, and a B chain, which is bound to the catalytic chain.The B chain contains two galactose-binding sites that bind to cell-surface glycolipids containing terminal galactose, facilitating the entry of the toxic A chain into the cytoplasm. After the A-chain is transported to the ribosome, the N-glycosidase in the A-chain acts to de-adenine the GAGA loop in 28SrRNA, thereby inhibiting the displacement of the polypeptide chain during protein synthesis, which inactivates the eukaryotic ribosome and allows cell death [10].

(3)Protein toxins of human origin

The use of biologically toxic proteins in the body, such as angiogenesis, neurotoxins and ribonucleases, as the active part of the immunotoxin. This helps to prevent the production of human antibodies against exogenous toxins (HATA) in the treatment, which makes it difficult to sustain the treatment [11].

(4)Dual or hybrid warhead toxins

Bacterial toxins and ribosome inhibitory proteins isolated from plants are used to prepare tumor-specific cytotoxic affixes. Since bacterial and plant toxins act at different steps in the translation process, combining their activities may be more effective. Thus, the double warhead immunotoxin is an immunotoxin prepared by cross-linking two different toxins with the same antibody. This immunotoxin is mainly coordinated with each other by the different intracellular transport pathways of the two toxins, and the ability to kill tumor cells depends on internalization, binding, and translational inhibition, and modulation of any of these can effectively increase the cytotoxicity of the immunotoxin [12].

Hybrid toxin (hybridtoxin), is a hybrid warhead molecule prepared through genetic engineering methods by recombining the genes of two different toxins in different ways. For example, using ricin A chain, diphtheria toxin, chimeric proteins were prepared by genetically fusing the coding region of ricin A chain and the A fragment of diphtheria toxin. The heterotrimeric proteins expressed in bacteria retained the N-glycosidase activity of ricin and the ADP-ribosylation activity of diphtheria toxin, resulting in better results than ricin or diphtheria toxin concatenates, in inhibiting tumor cell growth in vitro [12].

(5)T-cell based warheads

T cells are more conducive to infiltrating and destroying experimental tumors than other conventional immunotoxins. The chimeric expression of single-chain antibodies

with anti-tumor activity on T cells constitutes the so-called tumor-specific T-cell complex. It is capable of accurately recognizing tumor populations based on the single-chain antibodies chimerically expressed on its surface and exercising killing effects based on the activated T cells. This tumor-specific T-cell complex, overcoming the shortcomings of poor penetration of conventional immunotoxins, can infiltrate and effectively destroy solid tumors with excellent results, and has no obvious killing effect on normal cells [11].

3.2 Classification according to carrier molecules:

(1)Complete Monoclonal Antibodies

Conventional monoclonal antibodies are usually of murine origin and have a large molecular weight of about 150 KD.These antibodies have several advantages: strong antigenic affinity, long half-life, low cost, etc., and the immunotoxins prepared from them are mainly used in the treatment of hematologic tumors. However, they are rarely used in the treatment of solid tumors because of their difficult diffusion and penetration into the interior of solid tumors [11].

(2)single-chain antibody

The molecular weight is approximately 25KD, small, and consists of fragments of the variable region of the light and heavy chains of monoclonal antibodies linked by a connecting peptide. Compared to antibodies, the heavy chain variable region is the smallest antibody subunit that can mediate specific binding. The stability of the light and heavy chain FVs is maintained by adding linkers to the C and N termini of the FVs. This stabilized FV not only retains the specific binding ability, but also overcomes the disadvantage of intact monoclonal antibodies that are difficult to diffuse due to the large size of the molecule [9] [11].

(3)single domain antibody

With a molecular weight of only half that of a single-chain antibody, this single-domain antibody contains only the heavy-chain variable region V, yet retains most of the antigen-binding capacity of a single-chain antibody. It has the advantage of having a short half-life and is easily cleared in vivo [11].

(4)cell growth factor

Fusion proteins prepared by genetically engineering a truncated form of the toxin (PE or DT) to link it to a cDNA-encoded cytokine or growth factor, although they do not contain an antibody component and cannot be strictly defined as immunotoxins. However, its targeted import and killing mode of action against tumor cells is similar to that of an immunotoxin, enabling it to target numerous cytokine and growth factor receptors that are overexpressed on the surface of tumor cells. Certain ligands of cytokines and growth factor receptors can be used as carriers of immunotoxins, commonly including IL-2, IL-6, granulocyte/macrophage colony-stimulating factor (GM-CSF), and epidermal growth factor (EGF) [9].

(5)Humanized Antibodies

It is less likely to trigger an immune response while maintaining the affinity of the antibody, and has a long half-life in the body.

4. Major applications of immunotoxins in cancer therapy

Immunotoxins have become a focus of cancer immunotherapy research due to their unique ability to kill tumor cells. Immunotoxins, with their high specificity, low toxicity and high efficiency, have become a key point in cancer therapy.

(1)solid tumor

Immunotoxins are less effective in the treatment of solid tumors, but a variety of recombinant immunotoxins targeting solid tumors have been developed and applied, the e.g. Interleukin (IL)-4 and IL-13, ITIL13-PE38, Targeted mesothelin: SS1P, Anti-Louis Y immunotoxin, etc.

Interleukins [IL-4] and IL-13, two cytokines overexpressed in tumor cells, have been shown to be effective in vitro and in vivo, and thus have been used in targeted therapy for gliomas [23].ITIL13-PE38 exhibited potent cytotoxicity against renal cell carcinoma cell lines and was associated with ovarian cancer, Kaposi's sarcoma (KS), and squamous cell carcinoma head and neck cancer (SCCHN) were associated. Developed on the basis of an anti-melanoma antibody (scFvMEL), immunotoxins (Its) against tumor necrosis factor (TNF) demonstrated significant cytotoxicity in a nude mouse model [13]. Mesothelin, as a differentiated surface antigen, is highly expressed in a variety of tumors such as malignant mesothelioma, ovarian cancer, and pancreatic cancer, while it is only present in trace amounts in normal tissues, making it an ideal antigen for ligand-targeted therapy [14]. For the aforementioned cancers, PE38 was fused with SS1 (dsFv against mesothelin) to create SS1P (SS1[dsFv]-PE38), a recombinant immunotoxin with high affinity for mesothelin [24].

LewisY (LeY) antigen, a placental carbohydrate antigen, is highly expressed in a variety of epithelial cancers. Several immunotoxins have been developed against the LeY antigen using monoclonal antibodies MabB1 and MabB3 [15].

(2)Autoimmune disease-targeted Its.

A variety of immunotoxins for targeted therapy of autoimmune diseases have shown promising results in experiments.

e.g. DAB(389)-IL-2, AntiCD64-ricinIT, ITDT390-IP10-Sra, etc.

DAB[389]-IL-2 has shown promise in clinical trials for the treatment of psoriasis and rheumatoid arthritis [25].

In rheumatoid arthritis (RA), Anti-CD64-ricinIT is therefore effective in removing activated inflammatory synovial macrophages in vitro due to the high expression of CD64 (IgG receptor) in synovial macrophages and the ability of Itanti-FrβmAb-PE to target RA-specific synovial macrophages in vitro and in vivo [16].

ITDT390-IP10-Sra, containing interferon-inducible protein 10 (IP10), significantly reduced infiltrating CXCR3+ cells in experimental autoimmune encephalomyelitis (EAE) [16].

DT390-conjugated "Regulatory Activated Normal T Cells" (RANTES) are targeted to the CCR5 chemokine receptor [17].

(3)blood tumor

Many tumor cells present in the blood and bone marrow, often with high expression of surface markers, can be in stable and prolonged contact with drugs, making immunotoxin-based therapies more effective.

e.g. Denileukindiftitox (Ontak), anti-CD25 immunotoxin Bl22 (cat-3888).

DAB389IL2 was approved by the U.S. Food and Drug Administration (FDA) as the first FDA-approved immunotoxin. It is used for the treatment of recurrent cutaneous T-cell lymphoma (CTCL) [18], where the fusion protein targets the high-affinity IL2 receptor (IL2R) consisting of CD25 (IL2Ra), CD122 (IL2Rb), and CD132 (IL2Rg), which is overexpressed in a variety of malignant tumors (e.g., CTCL, ATL, HD, and other B/T-cell associated leukemias and lymphomas) and is also overexpressed in normal activated T cells and regulatory T cells [18].

The expression of CD25 (low affinity IL2 receptor) Is significantly higher than CD122 and CD132 (high affinity receptors) in most malignant tumors [19]. To target IL2R+ diseases expressing CD25, the higher affinity anti-Tac antibody was used in place of IL2 due to its specific binding to CD25.BL22, which is produced by fusion of the disulfide-stabilized anti-CD22 monoclonal antibody RFB4 (dsFv) with PE38, targets the CD22 molecule on the surface of B-cell malignant tumors (e.g., lymphomas, leukemias) [20]. Although normal B cells also express CD22, B-cell stem cells do not contain CD22, allowing normal B cells to be repopulated after BL22 treatment.

5. Conclusion

Immunotoxins have shown great potential in anti-cancer therapy. Many studies have pointed out that the effectiveness of immunotoxins relies on reducing their immunogenicity and prolonging the treatment cycle. However, its immunogenicity remains a significant problem in clinical trials. The unique anti-cancer mechanism of immunotoxins makes them ideal for cancer treatment and is expected to become an important tool for future cancer therapy.

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