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Research Article

Current status of immunotoxin application in cancer treatment

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Abstract:
Background and objective: Cancer is known as one of the most common causes of death in the world and the number of patients is increasing every year. Cancer is a major cause of death in the world.
Method: Due to the problems of using conventional methods of chemotherapy and radiation therapy, including the resistance of cancer cells to some chemotherapy drugs and having side effects and toxicity of these drugs on healthy tissues, it seems necessary to provide new solutions for specific and more effective cancer treatment. Immune glubloin-based treatments have become particularly crucial in the treatment of cancer. Today, the use of recombinant proteins that can track, identify and destroy specific tissues and cells is of interest to researchers.
Results: The result of the research was the discovery of a type of intelligent hybrid proteins called immunotoxins.
Conclusion: In this study we provided a review of the biological and clinical applications of immunotoxins in treatment of cancer. immunotoxins indicated that can act against cancer tissues. Immunotoxins might be effective in removing circulating tumor cells with large amounts of antigens.
Keywords: immunotoxins, cancer, cancer treatment, Ricin, diphtheria toxin, Pseudomonas

Background and objective

Cancer is known as one of the most common causes of death in the world and the number of patients is increasing every year. Conventional treatments are not only insufficient but also cause many side effects. Cancer is becoming the leading cause of death in most developed countries. In 2010, about 1.5 million new cancers were diagnosed in the United States, with an average death rate of 23%. To have a strong factor in suppressing cancer, that agent must directly and specifically target the cancer cell. Antibody-based treatments have become particularly important in the treatment of cancer, and so far, 28 drugs have been approved by the US Food and Drug Administration for the treatment of cancer[1,2]. In recent years, biological sciences have been involved in treating many diseases, including cancer. One of the most recently used of which is the use of Immunotoxins. Immunotoxins have been proposed as a novel compound in the treatment of cancer.

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Toxins are useful tools in basic biology and medicine. Immunotoxins are proteins that contain toxins and monoclonal antibodies or growth factor that bind specifically to target cells. So in this study we aimed to review the current aspects of the immunotoxins application in treatment of cancer.

Historical view point:

Initially, the importance of protein toxins was only considered as pathogens of bacteria or toxins eaten in poisonous plants. Years later, it was interesting to note that several of these toxins share common biochemical mechanisms, such as inhibiting protein synthesis. Diphtheria toxin and Pseudomonas exotoxin have similar mechanisms: they ADP ribose the Eukaryotic elongation factor 2 (EF-2) and inhibit protein synthesis during the elongation phase[3].

Several important researches in the late 1970s described the properties of immunotoxins in killing cells and shaped the future of this technology. A study in 1977 stated that diphtheria toxin had the potential to kill mammalian cells, and scientists later found that one diphtheria toxin molecule could kill one cell[4] . Therefore, the potential of diphtheria toxin and similar toxins was proven. Toxin potential depends on the turnover time and intracellular stability of the toxin enzyme region[5]. In another study, researchers introduced the use of antibodies to direct the lethal activity of toxins for specific purposes[6].

In particular, the use of anti-lymphocyte antibodies to kill lymphoblastoid tumor cells was assessed by chemical binding agents to bind diphtheria toxin to these antibodies, and so the primary immunotoxins were born[7]. The antibodies were then transformed into monoclonal antibodies, and immunotoxin molecules containing specific monoclonal antibodies of a specific chain (antigen epitope) were created. Then, for a while, the monoclonal antibodies were chemically attached to the toxins and new immunotoxins were produced (mostly for cancer treatment)[8]. The next step was to use molecular cloning methods that allowed the production of fusion proteins (immunotoxins) consisted of antibody fragments attached to toxin domains (enzymatically active)[9].

Mechanism of action

Although immunotoxins have good potential, other agents have been used along with immunotoxins for treatment[10]. Other areas of immunotoxins include the regulation of immune responses such as the removal of T cells from connective tissue or the removal of regulatory T cells and antiviral or antiparasitic activity[11]. Immunotoxin experiments on eukaryotic cells have led to the identification of new properties and functional domains of toxins[12].

The A chain of Ricin is a glycosidase and is toxic due to the deposition of a vital adenine in 28S rRNA. Researchers have come up with the idea for these toxins to make immunotoxins[13]. From the very first days, there were three candidate toxins: the plant toxin ricin (and other similar toxins expressed by other plants) and the diphtheria toxin and the exotoxin of Pseudomonas[14].

Factors influencing the selection of an appropriate immunotoxin include toxin structure, domains orientation, expression and purification efficiency, ease of cloning, glucose binding, immunization, and non-selective toxicity. Each toxin has a second enzyme activator and must reach the cytosol to kill the target cell. Each toxin also has a second binding that must be removed or neutralized before binding to the antibody[15].

Diphtheria toxin is transported to the cytosol by the T chain of the acidic endosome, while ricin and Pseudomonas exotoxin enter the rough endoplasmic reticulum before entering the cytosol. Pseudomonas exotoxin has specific sequences that help transport it to the endoplasmic reticulum[16]. Processing of diphtheria toxin and Pseudomonas exotoxin is performed with one step of protease breakdown and reduction of an important disulfide bond[17].

Binding of the toxin without the receptor region to the antibody produces the second-generation immunotoxin. Although a complete antibody (such as IgG) has a half-life and good performance in vivo, its large size limits the penetration of antibody into the tissue (especially in the case of solid tumors). In addition, most of the monoclonal antibodies used in the first and second generation of immunotoxins were of the monoclonal type of mouse antibodies. The use of non-human monoclonal antibodies has some disadvantages[18].

Molecular cloning methods, the production of fused genes (binding of genome fragments together in the laboratory) together with prokaryotic expression systems have revolutionized the production of immunotoxins and caused the production of third-generation immunotoxins[19].

The first significant development in the field of recombinant immunotoxins was the expression of single-chain fragments (antibody-variable region) in Escherichia coli, which they retained their antigen-binding properties. This finding led to the formation of the first recombinant antibody-toxin fusion proteins[20,21].

The third generation of immunotoxins are molecules containing variable antibody fragments and toxins without a binding chain. To date, more than 1,000 third-generation immunotoxins have been produced. Most of these immunotoxins selectively target antigens present on cancer cells. Although this approach was very attractive for the design of recombinant immunotoxins, there were several problems with the expression and purification of active monomer immunotoxins. Many of these problems were solved using codon optimization, inclusion body production, and other extraction methods[22]. Mesothelin molecules, CD22 and CD25 are antigenic candidates for the treatment of cancer in clinical trials. Other antigens, such as HER2/neu, Lewis-Y, CD30 and CD19, were used in preclinical studies but were left out due to systemic toxicity or poor cytotoxic activity against cancer cells[23,24].

Pseudomonas A exotoxin is one of the virulence factors of Pseudomonas aeruginosa. Pseudomonas exotoxin is one of the most common toxins used to treat cancer.

This exotoxin is a polypeptide chain that includes the following components: the N terminal region (Ia), responsible for cell attachment, the II region, responsible for transporting toxins through cell membranes, the exact role of region Ib is unknown but may be important in the secretion of toxins from bacteria. Zone III is the enzymatic portion of the toxin and has the activity of ADP ribosyltransferase[25].

Most recent advances in the production of immunotoxins have been in the production of smaller and less immunogenic types of PE40/38 molecules. With further deletion of the II domain, in PE of smaller molecules were produced that retained their cytotoxic activity and several immunogenic epitopes were removed. Removal of the II chain in PE toxin caused the removal of lysosomal cleavage sites and led to the production of a molecule called LR (due to lysosomal resistance). Thus, the LR type of PE-derived

immunotoxins had three characteristics: they were smaller, less immunogenic, and more resistant to enzymatic breakdown in lysosomes[26].

Regarding the binding of toxins to cell-binding ligands, the first candidate in the group of toxin-ligand molecules was EGF and then TGFα, IL-2, IL-4, IL-6, IL-3 ligands and in the case of diphtheria toxin, MSH, TF and IL-2 ligands have been used[27].

Toxin-ligand can be effective in killing cells through the receptor, but the toxin-ligand molecule has the potential to send different messages to cells. Many peptide ligands send growth or survival signals by binding to surface receptors. These messages are transmitted through phosphorylation cascades and they occur rapidly. Thus the growth or survival signals may be transmitted into the cell several hours earlier than the toxin. The toxin is then transported to the cytosol, inhibiting protein synthesis. Despite these concerns, the only approved immunotoxin in this group for treatment is DT-IL2, which is called Denileukin diftitox[28].

Diphtheria toxin is a 535 amino acid protein and is a type of ribozylating ADP toxins. This toxin is the only virulence factor of Corynebacterium diphtheria (diphtheria agent) and is similar in function to Pseudomonas exotoxin. Immunotoxin Denileukin diftitox or Ontac is the only immunotoxin approved by the US Food and Drug Administration, and the recombinant diphtheria immunotoxin, Anti-CD3 (A-dmDT390-bisFv(UCHT1)), binds to two scFvs (variable region) of an anti-CD3 mouse monoclonal antibody. In an experiment on six patients with CD3-containing T-cell lymphoma, the immunotoxin was found to kill more than 99% of normal T-cells over a two- to three-day period. In order to combine the drug-immunotoxin, the combination of ammonium chloride or Monensin with immunotoxin ricin A was also used to further sensitize the cultured cells and increase their effectiveness. Calcium channel blockers have also increased the activity of Pseudomonas exotoxin-based immunotoxins. Concomitant use of endosome-destroying adenovirus has also increased the activity of the pseudomonas immunotoxin. None of these compounds have yet been tested in living organisms, as it has been difficult to obtain the required levels of these drugs and there have been concerns about their safety[29,30].

Clinical aspects:

Immunotoxins have followed a predictable pathway for cancer treatment over 30 years of development. Then, clinical trials were designed and performed[31].

Various immunotoxins derived from the plant toxin ricin or bacterial toxins such as diphtheria toxin or Pseudomonas exotoxin have entered clinical trials[32].

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Types of Pseudomonas exotoxin-based immunotoxins include LMB-2 and OVB3-PE. LMB-2 is a third generation immunotoxin. This immunotoxin contains the VH and VL variable domains of mouse which VL is attached to PE38 (With the help of a connector called ASGGPE) at the C-terminus. CD25 is actually the alpha chain of the Interleukin 2 receptor (IL-2R). There is a large amount of IL-2 on the cells in immune disorders and blood malignancies. Phase I clinical trials for LMB-2 were completed in 2011. In this test, which was performed on patients with hematologic malignancies, out of 35 patients, one patient

responded completely to this immunotoxin (was treated) and seven patients responded partially[33,34].

Immunotoxin OVB3-PE contains IgG2b OVB3 monoclonal antibody of mice that is attached to the complete PE by a theatrical binding. OVB monoclonal antibody reacts with different cancer cell lines. In a clinical trial of 23 patients with ovarian cancer treated with OVB3-PE immunotoxin, none of the patients responded to treatment[35].

Immunotoxins, like other cancer drugs, have mild side effects such as diarrhea, fever, and nausea, and in some cases are severe, limiting the dose of immunotoxins used. Another disadvantage of immunotoxins is that they are immunogenic due to their protein structure, which triggers the reaction of the human immune system. The immune system produces antibodies against both the mouse antibody fragment and the protein toxin. Therefore, when the level of blood antibodies against immunotoxin is high, the patient can no longer receive immunotoxin treatment[36]. To solve this problem, a new generation of immunotoxins has been used: human or humanized antibody fragments, to reduce the immunogenicity of the immunotoxin. Another major drawback of immunotoxins is the development of vascular leakage syndrome. Immunotoxins normally are used intravascularly, so they come in contact with vascular epithelial cells, and this can cause vascular leakage syndrome. This syndrome can be severe and cause death. Other severe side effects of immunotoxins include hepatotoxicity. Another limiting factor for immunotoxins is their size. Therefore, by reducing the size of immunotoxins, their potential for entering solid tumors can be increased[37-39].

Conclusion:

Immunotoxins act selectively against cancer cells and have good potential for their detection and targeting. Immunotoxins may be the only effective drug in certain conditions, such as some circulating tumor cells with large amounts of antigen, but in most cases immunotoxins may be useful as a combination therapy with other agents. Toxin-based therapy is an extensive research field and can have wide applications in science and health.

Conflict of interests

None.

Authors' contributions

The authors are the same

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