Immunotoxins and Their Role in Prostate Cancer Treatment

Abstract

Considering the importance of immunotoxins in the treatment of patients with prostate cancer, this study aims to summarize the findings of preclinical studies related to the types of immunotoxins used in the treatment of prostate cancer and finally, the suggestions for the treatment of any of these diseases was better in the future. It seems that the important keys to success in the treatment with immunotoxins are the specificity of the target antigen and the internalization of the antigen-antibody complex to the cells that express the target antigens. Immunotherapy has long been important in advanced and treatment-resistant prostate cancer due to its anatomical location and sensitivity to immunotherapy agents. Among immunotherapy agents, satisfactory results have been obtained with monoclonal antibodies and scFv conjugated to toxins in preclinical studies. But so far, clinical trials with immunotoxins for prostate cancer have not been reported. Several antibodies that target antigens related to prostate cancer have been effective in preclinical and clinical development. Among these antibodies, anti-PSMA, PSCA, and epidermal growth factor receptor antibodies can be mentioned. Among the target antigens of prostate cancer, PSMA is known for its excessive expression and rapid internalization. It has become clear that both in active immunotherapy (vaccination) and in passive immunotherapy (monoclonal antibody), targeting multiple antigens simultaneously increases the efficacy of treatment in CRPC. Therefore, it is suggested that in future studies, several membrane-specific antigens of prostate cancer should be targeted by immunotoxins.

Keywords: Immunotoxins, Cancer, Prostate cancer, Prostate cancer treatment

Introduction

There were an estimated 1.3 million new cases of prostate cancer and 359,000 deaths worldwide in 2018, making it the second most common cancer and the fifth leading cause of cancer death in men.\(^1\)

The treatment of prostate cancer in different stages of the disease is different according to the grade of the tumor and the estimation of life expectancy. However, the ideal treatment for this disease has not yet been found and the effectiveness of treatment methods varies according to the progress of the cancer.\(^2\) Common treatments for patients with localized prostate cancer are radical prostatectomy, radiation therapy, hormone therapy, and cryosurgery.\(^2,4\)

Advanced prostate cancer is treated with chemotherapy and hormone therapy or androgen deprivation, which leads to the apoptosis of androgen-dependent tumor cells.\(^5,7\) However, after the initial response to this treatment, it causes androgen resistance in a large number of patients. In this case, prostate cancer enters its advanced stage, which is called androgen-independent prostate cancer (AIPC); because at this stage, the progression of cancer is independent of androgens, and therefore hormone therapy will be ineffective in patients. There is currently no effective treatment for AIPC. Chemotherapy mainly has palliative effects for patients with hormone-resistant prostate cancer, but in some cases, it shows survival effects.\(^8,9\) Therefore, new treatments and therapeutic goals in prostate cancer, especially for patients with high malignancy and advanced and extended types of prostate cancer (Castration-Resistant Prostate Cancer or CRPC), who have limited treatment options, are urgently needed.\(^10,11\)

New strategies for cancer treatment are based on designing drugs that specifically target cancer cells with minimal side effects in normal tissues. Monoclonal antibodies (Monoclonal antibodies or mAbs) are new groups of cancer treatments that can recognize target cells and specifically bind to the target cell.\(^12,13\) Today, 28 types of MAD have been approved by the Food and

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Drug Administration (FDA) for cancer treatment, and more than 100 types of MAD are used in different stages of clinical trials for cancer treatment.\textsuperscript{[14-16]} Despite the high efficiency of mAbs, these antibodies are rarely able to completely kill cancer cells. Different methods have been used to increase the productivity of antibodies, among them, binding to chemicals or toxins has had promising results.\textsuperscript{[17]}

Compared to mAbs, toxins show greater toxicity; so one molecule of poison is enough to kill a cancer cell. Therefore, immunotoxins can be a promising candidate for the treatment of prostate cancer in this type of treatment. Considering the importance of immunotoxins in the treatment of patients with prostate cancer, this study aims to summarize the findings of preclinical studies related to the types of immunotoxins used in the treatment of prostate cancer and finally, the suggestions for the treatment of any of this disease was better in the future.

\textbf{Immunotoxins}

Immunotoxins are formed from the binding of a toxin and a protein, which can be an antibody, a part of an antibody, or a growth factor. Immunotoxins get their potency from the toxin and their specificity from the antibody. Immunotoxins, with the help of their antibody part, identify the target cells bind to the cell surface proteins, and enter the cell with the help of the clathrin-covered membrane. The introduction of the toxin induces apoptosis in the target cell.\textsuperscript{[17-19]}

Immunotoxins have four generations. In the first generation of immunotoxins, the complete bacterial toxin is connected to the complete antibody through chemical bonding, which due to the presence of multiple binding sites in the toxin and antibody, the obtained immunotoxin is highly immunogenic and non-homogeneous and causes the death of cells. It becomes natural. For this reason, in the second generation of immunotoxins, the binding region of the toxin is removed so that it does not affect healthy cells. These recombinant toxins are attached to the complete antibody through a chemical bond. Although these immunotoxins bind specifically to the target cell, they are highly immunogenic for patients. Most of the monoclonal antibodies that were used in the production of the first and second generations of immunotoxins are of murine origin. Antigenic differences between known human and mouse antibodies, especially in the crystallizable fragment (FC) constant region, lead to an unfavorable immune response.\textsuperscript{[20-23]}

In the third generation, using recombinant DNA and genetic engineering principles, immunotoxins were created that contained only the necessary elements to identify and kill tumor cells. In fact, by removing the second (domain), identifying the target cell from the toxin, and replacing it with V (Variable fragment), an antibody, and then expressing it in a specific bacterium such as Escherichia coli, they were able to design the third generation of immunotoxins, which was more effective than the previous generations. The use of smaller antibody fragments as a targeting part is associated with a decrease in immunogenicity and an increase in the penetration of immunotoxins into solid tumors. For this reason, hundreds of third-generation immunotoxins have been developed so far.\textsuperscript{[24, 25]}

Due to the limited success achieved with modified toxins in animal models and clinical studies, efforts were made to replace the immunotoxin toxin with an endogenous protein of human origin. In the fourth generation, human antibodies were used to reduce the immunogenicity of the toxin, human antibodies are attached to internal cytotoxic proteins of human origin.\textsuperscript{[26]}

\textbf{Therapeutic immunotoxins}

\textbf{Immunotoxins in cancer treatment}

Denileukin difitotor (\textsuperscript{10}M ontalk) was the first immunotoxin approved for clinical use by the FDA. This immunotoxin is a chimeric protein that includes interleukin 2 and diphtheria toxin lacking the second binding (DAB389), which is used to treat TCL (Cutaneous T-cell lymphoma). Ontac binds to the interleukin 2 receptor present on the surface of leukemia cells including Hodgkin disease (ALT), Adult T-cell leukemia (HD), and CTCL, and exerts its effect.\textsuperscript{[27]}

DAB389EGF immunotoxin was formed by binding both enzyme domains and transferring DT (Diphtheria toxin) to a specific sequence of human epidermal growth factor (EGF) as the target part. DAB389EGF had an inhibitory effect on cancer cells overexpressing epidermal growth factor receptor (EGFR). This immunotoxin, which is in the preclinical stage for the treatment of bladder cancer, is also used to treat different types of glioblastoma in the model animal studies has been investigated and a direct relationship between sensitivity to DAB389EGE and the number of epidermal growth factor receptors on the surface of glioblastoma cancer cells has been observed.\textsuperscript{[28]}

\textbf{Immunotoxin in the treatment of prostate cancer}

Prostate cancer has characteristics that have become a suitable model for immunotherapy, among which it can be said that its specific antigens are well-known, and can provide suitable targets for the use of immunotoxins.\textsuperscript{[29]} Antigens targeted by immunotoxins include prostate specific-membrane antigen (PSMA) and prostate stem cell antigen (PSCA).

Prostate membrane-specific antigen is a type II membrane glycoprotein with an atomic mass of 100 kilodaltons, which contains the second membrane transmembrane, a small intracellular part, and an abundant extracellular domain. The expression of PSIA is androgen-independent, which increases with disease progression and reaches the highest level in CRPC. In addition, PSMA expression is abundant in tumor-associated new vessels, but not in normal vessels.

In addition, anti-PSMA radioimmunoconjugation has been investigated as an imaging agent as well as for cancer treatment. Therefore, PSMA is an ideal target for macromolecular mAb-based medicinal agents.\textsuperscript{[30]} Also, after reacting with antibodies, PSMA enters the cell, which can be used to deliver cytotoxic agents into the cell. It was used to target prostate tumor cells.\textsuperscript{[31]}

Accordingly, in the study of Fracasso et al., three monoclonal antibodies against (3591 PEQ226.5: PM2P079.1) were attached to the A chain of ricin, and their lethal effect was
In another study by Wolf et al., a recombinant immunotoxin (AS-PE40) composed of a single-stranded antibody fragment against PSA (CF) and a recombinant exotoxin A toxin of Pseudomonas aeruginosa (P40), which lacks the IA domain, was PSA expressing cells were studied. This immunotoxin was specifically bound to PSA-expressing cells and its half-minimal inhibitory concentration or IC50 reached 20 picomolar; while cells lacking PSA remained unaffected. Due to the high toxicity and specificity of this immunotoxin, this recombinant immunotoxin is a promising option in the field of treating prostate cancer patients. In the preclinical study of Wolf et al. D7-PE40 immunotoxin consisting of anti-PSMA antibody (produced through phage display from F11/3 monoclonal antibody) and exotoxin A on C4-2 cell line transfected PSA cells had increased expression, exposed to cells that D7-PE40 showed high stability in serum and caused a 50% decrease in IC50 in C4-2 cells at a concentration of 140 picomolar. Immunotoxin treatment on a 2-C4 tumor xenograft model in mice caused a significant inhibition of tumor growth, while in mice induced to develop tumors with DU145 cells (without PSA) were inoculated, it had no effect. According to these results, considering its cytotoxicity and its ability to prevent prostate tumor growth in vitro, D7-PE40 immunotoxin represents a promising option for prostate cancer immunotherapy.

In the study by Zhang et al., a bivalent immunotoxin produced by the fusion of a single-chain Diabody derived from the Fv fragment of an anti-PSMA monoclonal antibody with a short diptheria toxin (DT) fragment containing the activity and transport domains AdmDT390- scfDb (PSMA), which may be suitable for targeted therapy of PSA overexpressing tumors, was studied. In general, a bivalent immunotoxin containing two SCFV units has higher specificity and performance than SCF antibodies. In this study, PSA-positive and PSA-free cell lines were exposed to immunotoxin A-dmDT390-scD (PSMA). Cell uptake and selective toxicity of immunotoxin in cell culture of LNCap prostate cancer cells with PSA positive and It was negative in 3-PC prostate cancer cells lacking PSA.A-dmDT390-D (PMA) cell accumulation increased with increasing incubation time and concentration in INCaP cells, and the cell underwent apoptosis, which indicated a therapeutic and selective effect. Also, through optical imaging and MRI (Magnetic resonance imaging) of A-dmDT390-cfDb (PSMA attached to Alexa Fluor680) in xenograft tumors, they proved the specificity and therapeutic effect of this immunotoxin in nude mice.

Since Pseudomonas exotoxin-based immunotoxins sequentially inhibit the expression of the anti-apoptotic protein 1-Mc1, Noll et al. used the immunotoxin D7(VL-VH)-PE40, which was combined with 737-ABT to enhance the induction Apoptosis occurs in prostate cancer cells, they investigated and observed that the combination of this immunotoxin with 737-ABT causes enhancing effects or even synergism effect.

In another preclinical study conducted by Ma et al. PSLA monoclonal antibody was attached to tubulin polymerization inhibitor monomethylanurastin to evaluate the antitumor effect in vitro, and a xenograft model of mice with prostate cancer independent of Androgens was investigated. This conjugate destroyed PSMA-expressing cells with high potency and selectively, the survival rate of mice increased 9 times, and the effects of the treatment were also significantly reduced in the serum level of a prostate-specific antigen. More importantly, 40% of the treated animals had no detectable tumors or measurable PSA at day 500, indicating the therapeutic effect of this conjugate.

Many attempts have been made to reduce the immunogenicity of immunotoxins by humanizing/deimmunizing antibody binding domains, modification with macromolecules, or structural changes of toxin domains. The animal origin of some antibodies can lead to immunogenicity by stimulating the human response against immunotoxins. To overcome this limitation, many versions of less or non-immune antibodies have been developed using recombinant DNA technology, including chimeric, human, and fully human antibodies in different formats such as Fab, scFv, and DStv.

Another prostate surface marker that can be a suitable target for immunotoxins is prostate stem cell antigen (PSCA). This antigen is a protein with 133 amino acids that is located on the surface of prostate cells. This protein, with the help of glycosylphosphatidylinositol (Glyglycosylphosphatidylinositol or GPI), is attached to the cell membrane. This protein, which is considered a biomarker, belongs to the 1/Ly16-Thy family, and the reason for its name is that it has 30% structural similarity with type 2 stem cell antigen (2 Stem Cell antigen or SCAL) or surface marker. In humans, PSCA is expressed in the epithelial cells of the prostate, bladder, kidney, skin, esophagus, stomach, and placenta.

Studies have shown that this antigen is overexpressed in several common cancers such as renal cell carcinoma and bladder and pancreas cancers, and especially in 90% of prostate cancers. However, it has a very limited expression in healthy body tissues. This special expression pattern (difference of expression in cancerous and normal tissue) has caused PSCA to be considered a potentially useful and efficient biomarker in the prognosis, diagnosis, and treatment of prostate cancer. The results of research on PSCA indicate the fact that the expression of this cell surface marker is directly related to advancing the tumor stage and increasing the Gleason grade, invading the seminal vesicles and prostate capsule, and progressing to androgen-independent cancer. Therefore, in the advanced and extended type of prostate cancer, which has limited treatment options, its expression reaches its highest level, which can be a suitable candidate for targeted treatment.

In the study of Kessler et al. the recombinant immunotoxin consisting of PSCA (scFv which is derived from a human
monoclonal antibody, after the detection of the PSCA antigen in the HK cell line (Human embryonic kidney) in which PSCA was expressed, was used to The selected face entered the cell and had a lethal effect on the target cell.[43] Also, anti-PSCA monoclonal antibodies conjugated with Matamsinoid (a cytotoxic drug that inhibits microtubules) penetrate the tissue after detecting the antigen and have been very efficient in destroying tumor cells in vitro.[46, 47]

Results and Discussion

Immunotoxins act selectively against cancer cells and have a good potential to identify and target their antigens. Treatment based on immunotoxins is an extensive research field and can have wide applications in the field of cancer treatment. It seems that the important keys to success in the treatment with immunotoxins are the specificity of the target antigen and the internalization of the antigen-antibody complex to the cells that express the target antigens. Immunotherapy has long been important in advanced and treatment-resistant prostate cancer due to its anatomical location and sensitivity to immunotherapy agents. Among immunotherapy agents, satisfactory results have been obtained with monoclonal antibodies and scFv conjugated to toxins in preclinical studies. But so far, clinical trials with immunotoxins for prostate cancer have not been reported.

Several antibodies that target antigens related to prostate cancer have been effective in preclinical and clinical development. Among these antibodies, anti-PSMA, PSCA, and epidermal growth factor receptor antibodies can be mentioned. Among the target antigens of prostate cancer, PSMA is known for its excessive expression and rapid internalization. In past studies in the field of immunotoxin therapy, they have mainly targeted the PSMA antigen, which is abundantly found on the surface of prostate cancer cells. It has become clear that both in active immunotherapy (vaccination) and in passive immunotherapy (monoclonal antibody), targeting multiple antigens simultaneously increases the efficacy of treatment in CRPC. Therefore, it is suggested that in future studies, several membrane-specific antigens of prostate cancer should be targeted by immunotoxins.[48-51] However, this method requires the discovery and disclosure of new prostate cancer-specific surface antigens that also have the power to internalize immunotoxin.

It has been reported that immunotoxin treatment induces a host immune response against the heterologous toxin and leads to a reduction in the half-life and neutralization of the cytotoxic potential of immunotoxins.

Toxins used in immunotoxin therapy in prostate cancer are mainly of bacterial origin. Although bacterial toxins are considered strong toxic agents for tumor cells, bacterial toxins are highly immunogenic in humans and strongly stimulate immune responses. However, if we use parts of the toxin with weak immunogenicity, they may not be effective enough to destroy tumor cells. Several previous studies have shown that deletion or mutation in the second toxin leads to the removal of T-cell and B-cell epitopes and successfully leads to reduced immunogenicity and reduced production of immune response. On the other hand, the high immunogenicity of bacterial toxins can intensify inflammation, and as some studies have shown, there is a direct relationship between inflammation and prostate cancer progression, and it causes changes in the tumor microenvironment.[52-54]

Conclusion

This study suggests that for the best treatment control of prostate cancer patients in the foundation of future in vitro and clinical studies, androgenic toxins such as granzymes along with targeting several tumor antigens should be investigated and studied at the same time.

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None.

Conflict of interest

None.

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Ethics statement

None.

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