Tumor Immunity as Well as Its Treatment Methods

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Abstract: in the past years, tumor immunotherapy has become a major research topic and up till now, some remarkable progress has been achieved. It has come up with a new oncotherapy method different from surgery, conventional chemoradiotherapy. Tumor immunotherapy aims to kill or inhibit tumor growth by activating the immune system of the human body itself or enhancing the functions of the immune system. This thesis reviews the research and development of oncolytic virus therapy, CAR-T therapy and phytochemical drug therapy.

1. Introduction
Tumor is a malignant disease caused by accumulation of gene mutation in normal somatic cells, which results in uncontrollable cell proliferation and division and failure to complete normal cell apoptosis. Tumor immunity is the body's own protective system against tumors, including specific and non-specific immunity of T lymphocytes, B lymphocytes, natural killer cells, macrophages, antibodies, complement, cytokines, etc. Under the protection of the immune system, tumors live and proliferate in the body through tumor immune escape, which exerts adverse effects on the body's health and immune function. Tumor immunity is a science that studies the antigenicity of tumor, the immune function of the body, the relationship with tumor, and carries out diagnosis and treatment of tumor immunity on the basis of the above.

Tumor Immunotherapy

2. Oncolytic Virus Therapy

2.1 What is Oncolytic Virus
Oncolytic virus (T-Vec) is a kind of virus. It replicates in tumor cells and lyses tumor cells principally by means of different regulatory mechanisms, but does not affect the growth of normal cells [1], including Newcastle disease virus, oncolytic adenovirus, herpes simplex virus-1, reovirus, etc. In the process of lysing tumor cells, oncolytic virus releases specific tumor antigen to activate specific immune response of the body, and finally achieves the goal of killing tumor cells and protecting human health [2].

2.2 Mechanism of Action of Oncolytic Virus
Oncolytic virus was developed by AMGEN Company in the United States, and on the basis of HSV-1, it is modified from JSI virus therein. However, it does not contain γ34.5 gene and α47 gene in HSV-1. In the meanwhile, human granulocyte-macrophage colony-stimulating factor (GM-CSF)[3] was implanted at γ34.5 site. In normal cells of human body, the virus replicates principally through proliferation of nuclear antigen (PCNA)-γ34.5 complex, but DNA repair protein and PCNA in tumor cells are highly expressed. Hence, the γ34.5 gene in HSV-1 in the body is deleted, selectively
replicated in tumor cells, lyses tumor cells and causes them to die, simultaneously releases progeny virus, continuously infects tumor cells in tissues and realizes repeated killing [4]. The α47 gene principally inhibits the antigen presentation related proteins to prevent the virus from playing the role of antigen presentation. After eliminating the α47 gene, oncolytic virus can stimulate the formation of specific immune response and achieve the goal of optimizing oncotherapy effect [5]. Apart from that, oncolytic virus can also integrate GM-CSF coding sequences that facilitate the maturation of DC and macrophages, further enhance the function of tumor antigen, and transfer it to CD8+ T cells with specific immune function, finally forming potential anti-tumor immunity.

2.3 Advantages of Oncolytic Virus over Other Therapies
In comparison with other therapies, oncolytic virus boasts multiple advantages, such as more virus types, more regulation methods, etc. It can serve as a vector to realize full expression of foreign genes. In the meanwhile, oncolytic virus can also be integrated with tumor immunotherapy to recruit tumor infiltrating lymphocytes and enhance tumor specific immune response, laying a foundation for effective development of other therapies. With respect to the route of administration, oncolytic virus can be injected directly into tumor for administration, which can enable HSV-1 live virus to enter tumor tissue, infect tumor cells efficiently, and cause tumor cells to lyse and die. Moreover, the lysed tumor cells can release tumor antigen, stimulate specific immune response and enhance anti-tumor effect. Studies have verified that oncolytic virus therapy, as a selective tumor killing biological drug, can significantly enhance the arming of immune factors, and realize the dual effects of cracking tumor cells and upgrading the immune ability of the body tumor. Furthermore, oncolytic virus can also be used in combination with chemoradiotherapy and operative treatment to maximize the effects of these therapies [6].

2.4 Clinical Application of Oncolytic Virus

2.4.1 Individual Involvement of Oncolytic Virus in Anti-tumor Therapy
Oncolytic virus is fully involved in multiple immunotherapeutic stages of tumor cells. It can effectively kill tumor cells by lysing tumor cells, expressing foreign genes, presenting infected tumor cells as new tumor antigens, promoting the accumulation of infiltrating CD8+ T lymphocytes in tumor microenvironment, and stimulating the body to form specific tumor immune function. In a healthy human body, cells have a complete anti-virus mechanism, and can accurately identify DNA viruses and RNA viruses by using recognition factors such as NLRP3, oligoadenylate synthetase, PKR, Toll-like receptor 3, Toll-like receptor 7, retinoic acid-induced genes, etc., so that interferon -I can be expressed to eliminate viruses in a prompt manner, CD8+ T lymphocytes can be recruited timely, and the expression ability of MHC-I molecules can be upgraded [7]. And in tumor cells, the antiviral ability of the cells is generally deficient, and oncolytic viruses can be directly replicated in tumor cells. Influenced by the oncolytic effect of oncolytic virus, tumor cells release pathogen-related molecules and cell-derived damage-related molecules, induce an increase in antigen immune response, facilitate MHC-I molecule expression, enhance the recruitment effect of BATF3+DC cells, and jointly exert antiviral immunity. Hence, oncolytic virus has been used alone in anti-tumor therapy in clinical practice with remarkable curative effect.

2.4.2 Combination of Oncolytic Virus with Chemoradiotherapy to Enhance Anti-tumor Effect
Chemoradiotherapy can directly kill tumor factors, but cannot act on the pathological mechanism of tumor virus. The combination of oncolytic virus with chemoradiotherapy can play a synergistic role with oncolytic virus through radioactive rays of chemoradiotherapy, thus significantly enhancing the oncolytic effect of oncolytic virus. Besides, under the living body imaging technology, the virus distribution and gene expression can be accurately determined, and the clinical curative effect can be judged more accurately. Reovirus in oncolytic virus can play a targeted therapeutic role and enhance the immune characteristics of tumor cells. Radiographic exposure can further activate Ras pathway
without affecting the replication behavior of oncolytic virus in tumor cells. Sodium iodide symporter (NIS) is a transmembrane glycoprotein, which is expressed in the basement membrane of thyroid follicular cells to accumulate iodine [8]. Currently, NIS gene has been extensively used in all sorts of tumor models. It is characterized by small trauma and high accuracy, and can help to track virus replication, which has obvious enhancement effect on targeted therapy of tumor cells.

2.4.3 Combination of Oncolytic Virus and Immunotherapeutic Drugs to Enhance Anti-tumor Effect

Clinically, programmed death receptor 1 (PD-1) and programmed death ligand 1 (PD-L1) blocking agents are commonly used to enhance immune cell activity, block tumor cell division, activate immune function, and achieve anti-tumor purposes. However, this blocking agent cannot play a role in tumors that are not expressed or are not obviously expressed by tumor cells. Oncolytic virus is used in combination with PD-1 and PD-L1 blocking agents to enhance the specific immunity of tumor cells, generate immune cells and realize tumor infiltration through the induction of oncolytic virus on tumor cells. While in the face of massive invasion of immune cells, tumor cells once again increase the expression of PD-L1, enabling PD-1 and PD-L1 blocking agents to discover tumor cells in a timely manner and form blocking effect, thus expanding the blocking range. Robert H.I [9] et al. have conducted research on mouse lymph cancer transplantation tumor model, which proves that the combination of vaccinia virus and α-PD-L1 blocking agent in oncolytic virus can significantly improve the anti-tumor effect. Hao Mengru, Huang Chenghao [10] and other clinical trials also demonstrate that the tumor remission rate of melanoma patients can be up to 84.3% by blocking tumor cells with PD-1 blocking agent and combining with oncolytic virus therapy, which is significantly different from that of patients using PD-1 blocking agent alone (60.37%). Apart from that, the study also discovers that the expression of PD-1 and PD-L1 is more obvious when the immune cells infiltrate tumor cells. All these studies have proved that the combination of oncolytic virus and immunotherapeutic drugs can improve the microenvironment of tumor tissues and enhance the therapeutic effect of blocking agents.

3. Chimeric Antigen Receptor T Cells Therapy

Chimeric antigen receptor T cells (CAR-T) therapy is one kind of adoptive immunotherapy. It constructs specific chimeric antigen receptor (CAR) in vitro by artificial method, uses chimeric gene of transgenic technology to make T lymphocyte express CAR, then amplifies these cells in vitro, and finally sends them back to patients for treatment. CAR can specifically recognize target antigen and kill tumor cells in vivo. The structure of CAR consists of three parts: extracellular domain, transmembrane domain and intracellular domain. The main components are as below: single chain variable fragment (ScFv), CD3-ζ chain or intracellular segment of FcεRIγ, intracellular segment of costimulatory molecules (CD28, CD134).

3.1 Advantages of CAR-T Therapy

Cytotoxic T cells (CTL) need to recognize MHC when recognizing tumor cells, and are limited by human leukocyte antigen molecule (HLA). CAR-T boasts the following advantages over CTL: (1) the reformed CAR-T cells are not restricted by MHC in the process of tumor recognition, recognize tumor antigens in an HLA independent manner, and recognize and kill tumor cells across the HLA expression down-regulation mechanism; (2) CAR utilizes the diversity of antibodies to recognize not merely protein antigens on tumor surface, but glycan and glycolipid antigens in glycoprotein, with more tumor antigen targets to choose from; (3) CAR-T cells can survive for a long time in vivo and have long acting time; (4) Using patients’ own T cells to construct CAR-T cells in vitro reduces the risk of rejection; (5) CAR-T therapy has obvious therapeutic effect on blood tumor.

3.2 Defects of CAR-T Therapy

CAR-T therapy still has some defects in clinical application, specifically as below: (1) due to the heterogeneity of tumors, the complex tumor microenvironment of solid tumors, the homing ability of
CAR-T, etc., CAR-T therapy is lack of substantial progress in solid oncotherapy; (2) CAR-T input may make cytokine release syndrome, causing systemic inflammatory response, systemic organ dysfunction and even threatening the life; (3) low expression target antigen in normal tissues may also be attacked by antigen-specificity of CAR-T cells, resulting in off-target effect. Up to now, no precise tumor surface-related antigen has been found, resulting in harmful activation of CAR-T; (4) tumor cells can inhibit the activity of immune cells by a variety of ways, making immune system in tumor microenvironment in a tolerant state, resulting in the patient's own immune response unable to inhibit tumor growth; (5) Due to CAR-T's strong tumor cell killing ability, cells dissolve rapidly and intracellular substances are released into the blood in large quantities, exceeding the metabolic capacity of kidney and liver, resulting in neurotoxic symptoms of the body.

3.3 Clinical Application of CAR-T Cell Immunotherapy

3.3.1 T Cell Lymphoma
Since there also exist therapeutic targeted antigens on the surface of normal T cells, it is relatively difficult to apply CAR-T technology to T cell lymphoma. CAR-T technology targeting CD7 through CRISPR/Cas9 gene editing technology has been tried to be applied to T cell lymphoma, and certain anti-tumor effect has been found in preclinical studies [11].

3.3.2 Malignant Melanoma
Malignant melanoma is a tumor produced by melanocytes of skin and other organs. Despite that its incidence is low, it is subject to high malignancy, early metastasis, high mortality and poor prognosis. Even if radical surgery is performed in the early stage, the survival rate of patients is low as well. Thus, CAR-T cell immunotherapy may be a breakthrough in the treatment of this disease.

Melanoma carries a large number of mutant genes, and tumor antigen expressed by it can be recognized by the specific immunity of patients, so this kind of antigen can be employed as a targeted antigen for CAR-T therapy to make it effective.

3.3.3 Neuro Blastoma
Neuro blastoma (NB) is a neuroendocrine tumor, which can originate from any nerveous part of sympathetic nervous system. The most common occurrence part is adrenal gland, but it can also occur in nerve tissues of neck, chest, abdomen and pelvic cavity. For infants under 2 years old, the malignancy degree is high and the prognosis is poor. Despite that the overall survival rate of child patients increases along with the development of chemotherapy and radiotherapy, etc., the prognosis of high-risk child patients is still poor, so CAR-T may become a new treatment option.

Ganglioside GD2, as a tumor-related carbohydrate antigen, is associated with tumor proliferation, spread, angiogenesis and metabolic immunity. Studies have revealed that GD2 is expressed in high density in neuroblastoma tissues and low density in other neurogenic cell tumors, so GD2 can be regarded as the best target for CAT-T therapy.

Phytochemical Drug Therapy

4. Sulforaphane
Sulforaphane (SFN) is an isothiocyanate existing in cruciferous plants, and it is quite abundant in broccoli, cabbage mustard, carrot and other cruciferous plants. The content of sulforaphane in broccoli is the highest [12], because glucosinolates exist in cells of cruciferous plants. When plants are damaged, glucosinolates can be hydrolyzed by myrosinase to produce homologs and isothiocyanates. The chemical formula of sulforaphane is CH3S(O)(CH2)4-N=C=S. Sulforaphane is a natural plant chemical for tumor prevention, and it can prevent the occurrence of tumor, inhibit the growth of tumor cells and induce the apoptosis of tumor cells through various ways.
4.1 Possible Mechanism of Sulforaphane Inhibiting Tumor
Sulforaphane has a specific inhibitory effect on tumor stem cells, is easy to pass through the blood-brain barrier without obvious adverse reactions, and can inhibit all sorts of tumor stem cells such as breast cancer, lung cancer, gastric cancer and the like [13]. The following is the possible mechanism of action of sulforaphane: (1) SFN is the most potential phase II enzyme inducer. SFN exerts its antioxidant effect by inducing phase II detoxifying enzymes (such as glutathione sulfurtransferase, benzoquinone reductase, epoxide hydrase and UDP-glucurononyltransferase [14]), and does not directly react with free radicals or reactive oxygen species. Hence, SFN is an indirect antioxidant substance. In the absence of SFN, its indirect antioxidant effect can still last for several days; (2) the apoptosis of HT-29 cells caused by FSN is associated with the overexpression of bax, which causes cell death in bcl-2 gene family. Bax is a cytoplasmic protein that can enter mitochondria to exert its effect, and can cause mitochondria to release cytochrome C, and finally activate Caspase cascade reaction to cause apoptosis [15]; (3) SFN can down-regulate the expression of COX-2 in mouse macrophages. The up-regulation of COX-2 expression is closely associated with the occurrence of tumors. SFN principally inhibits the transcription of tumor cells by inhibiting the activity of NF-κB to inhibit LPS-induced gene expression; (4) SFN is lipophilic and can induce cell growth arrest and apoptosis by inhibiting biosynthesis of phospholipids and other lipid substances.

5. Hydroxycamptothecine
Camptothecin (CPT) is an alkaloid extracted from the seeds or roots of camptotheca acuminata of Davidia involucrata. CPT can inhibit DNA synthesis, inhibit the growth of tumor cells, but do too much damage to urinary system. Hydroxycamptothecin (HCPT) is a hydroxyl derivative of camptothecin, which is synthesized by substituting -OH for -H in ring A of camptothecin basic ring. Hydroxycamptothecin has the same anti-tumor effect as camptothecin, with less side effects, reliable clinical efficacy and high safety [16].

5.1 Possible Mechanism of Hydroxycamptothecine Inhibiting Tumor Growth
The possible mechanism of HCPT is to irreversibly inhibit DNA replication and cleavage of replication forks. CPT can act on Topo-I, untwist DNA double strands and temporarily break one strand. When replication reaches the site of DNA cleavage, replication fork is blocked and cannot move forward. CPT stable TopoⅠ-DNA cleavage complex can change the local DNA structure of replication fork, and the cleavage complex cannot be recovered after removing drugs [17]. These drugs are not cross-resistant to other commonly used anticancer drugs, so they have good therapeutic effects on drug-resistant tumors [18].

5.2 Scope of Action of Hydroxycamptothecine
Hydroxycamptothecine is principally used for cancers of digestive system, such as gastric cancer, intestinal cancer, esophageal cancer, liver cancer and the like, and produces fairly good curative effects on nasopharyngeal carcinoma [19], bladder cancer, lung cancer and clinical effects on other cancers [20]. Furthermore, hydroxycamptothecine may be used for anti-inflammatory and septic treatment [21].

6. Summary and Prospect
Currently, tumor immunotherapy has become the first choice for clinical treatment of tumors. Traditional surgery can only be employed to remove local tumors and is not suitable for malignant tumors and hematological tumors that have undergone extensive metastasis. The adverse reactions brought by traditional chemoradiotherapy are too large. By contrast, tumor immunotherapy boasts better curative effect, less adverse reactions and is closer to the needs of patients.

Tumour immunotherapy does bring better treatment options for tumor patients. The combination with immunotherapy also solves some deficiencies of monotherapy, but there still exist great challenges. For instance, individual differences are relatively large, and each patient needs
individualized treatment strategies to realize individualized precise treatment; how to overcome the off-target effect of CAR-T therapy and cell storm problem; there is no guarantee that all tumor immunotherapy is clinically effective for all tumors.

It is believed that thanks to the development of tumor immunology and further research on target spots in tumor microenvironment and tumor metabolism, an increasing number of effective and low-toxicity anti-tumor drugs will enter clinical treatment, offering more choices for oncotherapy.

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