The development and application of therapeutic tumor vaccine

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Abstract

Effective, safe, and enduring tumor treatments constitute major challenges of modern medical sciences. Now, with the unprecedented success of anti-PD1/PD-L1 checkpoint inhibitor antibodies and CAR-T cell therapy, tumor immunotherapy represents a very promising way to cure cancers. Therapeutic tumor vaccines represent an attractive treatment modality for tumors by stimulating patient's own immune system to evoke a long-lasting protective antitumor immunity. Numerous vaccine strategies are currently under development or being evaluated both preclinically and clinically. Tumor vaccines can be classified into several major categories, which include dendritic cell-based vaccines, peptide vaccines, and nucleic acid vaccines (including DNA and RNA vaccines). mRNA tumor vaccine, especially the recently-developed neoantigen mRNA vaccine is an effective, specific and safe weapon to activate patient's own immune to battle with tumors. In this review, we focus on different type of tumor vaccines, summarize their recent advances and point out the future direction to develop new and better vaccines to fight with cancers.

Keywords

Tumor vaccine, immunotherapy, dendritic cells, RNA, neoantigen.

1. Introduction

For centuries, human beings have developed many strategies based on the ever-increasing understanding of tumor biology to cure cancers. The treatment-target among the majority of strategies is tumor cell itself, but the clinical results are always far from satisfactory. Tumor immunotherapy is different from conventional therapies, the main aim of tumor immunotherapy is to stimulate patient's own immune system to attack tumor cells and improve clinical outcomes [1]. In recent years, researches developed many different classes of immunotherapies to boost the anti-tumor immune responses, which include cytokines, immune checkpoint inhibitors, adoptive T cell therapies (including TIL therapy, chimeric antigen receptor (CAR) T cells and TCR-T cell therapy), and tumor vaccine strategies [2-4]. Several of them, such as the anti-PD1/PD-L1 immune checkpoint inhibitors and anti-CD19 chimeric antigen receptor (CAR) T cell therapy in B-cell leukemia, have demonstrated impressive survival benefits to patients [5, 6]. In 2014, FDA approved two PD-1 blocking antibodies, Pembrolizumab (Merck) and Nivolumab (Bristol-Myers Squibb), for use in patients with advanced or unresectable melanoma who fail to respond to other therapies, which represent the beginning of a new era for cancer immunotherapy. In the following three years, Nivolumab was approved for treatment of squamous lung cancer, Hodgkin's lymphoma and FDA approved PD-L1 blocking antibody Atezolizumab (Roche) to treat the patients with bladder carcinoma. In late 2017, CTL019 (tisagenlecleucel), a CD19-targeting CAR T-cell therapy developed by Novartis for treating relapsed or refractory B-cell acute lymphoblastic leukemia (ALL) in children and young adults was approved by FDA.

Compared with the other types of cancer immunotherapy, tumor vaccine therapies are considered to be a type of specific, safe, and well-tolerated cancer treatment, they have the potential to avoid drug resistance and obtain durable treatment responses due to the long-term immunologic memory [7]. Dendritic cells are the most potent professional antigen-presenting cells (APCs). They uptake, process and present pathogen- or host-derived antigenic peptides at peripheral tissues, stimulate naive T lymphocytes at the lymphoid organs in a major histocompatibility (MHC) molecules-dependent way. Boosting the ability of dendritic cells to acquire and process tumor antigens, potentiate the host

anti-tumor immune response and increase the breadth and diversity of tumor antigen-specific T cells lies at the core of tumor vaccine therapies [8]. Two key factors may greatly influence the effect of tumor vaccine, the first is to find the appropriate tumor antigens, and the second is to use the right agent to immunization [9, 10]. For the first question, the validity of a target for therapeutic cancer vaccine mainly depend on its specificity for tumor versus normal adult tissue. Common vaccine targets have been test in clinical or animal model include various tumor associated antigens (TAAs, such as oncofetal antigens, oncoproteins, differentiation-associated proteins), viral proteins and proteins with somatic point mutation [11]. In this review, we mainly focus on the second question, summarize the recent advances, the pros and cons of different vaccine methods, and point out the future direction to develop new and better vaccines to fight with cancers.

2. DC-based vaccines

DCs are important sponsor to mount a robust antigen-specific immune responses. Directly pulse tumor antigens to DCs represents a simple and efficient way to develop therapeutic tumor vaccine [12].

The Sipuleucel-T (Provenge) was approved by FDA in 2010 for the treatment of asymptomatic metastatic castrate-resistant prostate cancer (mCRPC). This autologous vaccine mainly consists of DCs from PBMCs that have been incubated *in vitro* with PA2024, a recombinant protein that fuse prostatic acid phosphatase (PAP, is an enzyme highly expressed by the prostate) with GM-CSF. In theory, after infusing back to patient, Sipuleucel-T will specifically activate T cells bearing TCR that recognize epitope derived from PAP, then these CTL cells will attack prostate cancer cells. Clinical results showed that a survival advantage over control group was achieved [13]. In view of its favorable toxicity profile and manageable route of administration, the success of Sipuleucel-T as the first therapeutic cancer vaccine opens a new door for prostate cancer and other cancers.

Sipuleucel-T vaccine only targets to the PAP antigen, it would be highly desirable that such antitumor responses include multiple T cell clones against multiple tumor antigens. In a phase I/IIa clinical trial, Greene et al. developed an autologous tumor-dendritic cell fusion (dendritoma) vaccine with low dose IL-2 to treat stage IV melanoma [14]. Theoretically, the full repertoire of tumor antigens from an individual's cancer can be uptaken, processed and present [15]. Although this initial trial was small, this vaccine produced a median survival of 16.1 months and the 5-year survival of 29.2 %, a vast improvement over historical controls and a clinical benefit that is similar in magnitude to checkpoint inhibitor therapy.

Berard and colleagues demonstrated that DCs pulsed with autologous killed melanoma cell can induce specific T-cell responses against several melanoma-specific TAAs, including MART-1 and GP100 [16, 17]. In another study, Wang and colleagues demonstrated bone marrow-derived DCs pulsed with Hepa1-6 cell lysates generated from multiple freeze/thaw cycles followed by maturation with lipopolysaccharide (LPS) prevented HCC progression in a clinically relevant murine HCC model [18].

In 2015, Carreno and colleagues demonstrated that neoantigen peptides pulsed DC vaccine induce a robust anti-tumor immune responses and broaden the clonal diversity of CD8 TCR in melanoma patient [19]. This is also the first proof of concept study in human to show the prowess of neoantigen vaccine in antitumor immunity.

3. Peptide-Based Cancer Vaccines

The availability of patient's autologous tumor cells and DCs, and the complex procedure of preparing individualized vaccines greatly limit the broad use of dendritic cell based cancer vaccines. Peptides from tumor-associated antigens (TAAs) formulated with adjuvant or immune modulator as tumor vaccine clearly have several advantages over DC vaccines. Most peptide-based vaccines in clinical trials target cancer-testis antigens (such as MAGE and NY-ESO-1), differentiation-associated

antigens, or certain oncofetal antigens (CEA, MUC-1). Previous clinical outcomes based on this method are disappointing, but researchers achieved encouraging results recently [20].

Kenter and colleagues investigated the efficacy of a synthetic long-peptide vaccine against the HPV-16 oncoproteins E6 and E7 in women with HPV-16-positive, high-grade vulvar intraepithelial neoplasia. Twenty patient were vaccinated up to four times with a mix of long peptides and incomplete Freund's adjuvant. At 12 months of follow-up, 15 of 19 patients had clinical responses, with a complete response in 9 of 19 patients. The complete response was maintained at 24 months of follow-up [21].

In 2017, ott and colleagues demonstrated that vaccination with neoantigen peptides in melanoma patient can both expand pre-existing neoantigen-specific T-cell populations and induce a broader repertoire of new T-cell specificities, tipping the immune balance in favor of enhanced tumor control [22, 23]. Through whole-exome sequencing and bioinformatics prediction, 20 peptides (with lengths of 15-30 amino acids) containing predicted mutation per patient were chemically synthesized, admixed with poly-ICLC, and administered subcutaneously. Results showed that these peptide vaccines induce polyfunctional CD4+ and CD8+ T cells targeting to tumor cells. Of six treated patients, four had no recurrence at 25 months after vaccination, while two with recurrent disease were subsequently treated with anti-PD1 mAb and experienced complete tumor regression [24].

4. mRNA Vaccines

Another strategy to deliver and express tumor antigen is to utilize DNA or messenger RNA (mRNA) that encoding tumor specific antigen [25]. Compared with DNA, RNA has many advantages. First, RNA only needs to gain entry into the cytoplasm, and can be translated immediately in the host cells. Second, RNA cannot integrate into the genome and therefore has no oncogenic potential. Third, RNA can stimulate toll-like receptors and act as an adjuvant by providing costimulatory signals [26]. So, there is a growing interest in the research and development of RNA tumor vaccines.

mRNA vaccines can be generated by in vitro transcription (IVT) using a bacteriophage T7 RNA polymerase from DNA template. mRNA stability and translational efficiency are of vital importance to the production of antigen protein and the degree of anti-tumor immune responses generated. But naked mRNA is easily degraded by the ubiquitous ribonucleases, which greatly impede its applications [27]. Through continuous improvement and development, now modified IVT mRNA with great stability and translational efficiency is successfully applied to tumor patients [28]. Just as the mimic of native eukaryotic mRNA, the most potent IVT mRNA vaccine is composed of a coding region flanked by beta-globin untranslated regions (UTR), 5′ anti-reverse cap analogues (ARCA) and 3′ 120-150nt poly(A) tail [29].

In dendritic cells, mRNA-encoded proteins are degraded by proteasomes and presented on MHC class I molecules to prime CD8+ T cells, but not reach lysosome and the MHC class II pathway to induce CD4+ T cell responses. Then, researchers found that addition of a lysosomal targeting signal, such as LAMP-1 and DC-LAMP, or MHC class I trafficking signal (MITD) to the end of antigen-encoding sequence can result in both HLA class I and II presentation, and the expansion of Ag-specific CD8+ and CD4+ T cells and improved effector functions [30, 31].

Different administration routes also influence the vaccination effects [32]. Kreiter et al. reported that intranodal vaccination using naked antigen-encoding mRNA induces robust CD8+ and CD4+ T cell responses and superior antitumor immunity, compared with other application routes [33]. Intranodal injected RNA can be specifically acquired by lymph node resident dendritic cells (DCs) with higher efficiency than subcutaneous injection.

In 2017, Ugur Sahin and colleagues adopted mRNA based individualized neoepitope vaccine to activate immunity against a spectrum of cancer neoantigen in melanoma patient [34]. Through next-generation sequencing technology to identify somatic mutations and computational prediction of neo-epitopes, they designed and manufactured mRNA vaccines consist of up to 20 neoantigens per patient. These RNA vaccines that without adjuvant were directly injected into inguinal lymph nodes

under ultrasound assistance. Vaccine induced an unexpectedly broad repertoire T cell immune responses against multiple neoepitopes in all treated patients, and remained recurrence-free for the whole follow-up period (12 to 23 months) [35].

It is noteworthy that due to the high polymorphism of HLA alleles and the complexity of peptide-MHC binding selection, accurate epitope prediction and selection is vital to the future success of this personalized neoantigen vaccines.

5. Conclusion remarks

Therapeutic cancer vaccines are designed to induce durable antitumor immunity that is to protect patient against tumor recurrence and metastasis. Many types of therapeutic cancer vaccine have been explored, with varying levels of success, especially the recently-developed neoantigen vaccine strategy [36]. Combining vaccine strategies with other approaches, such as checkpoint inhibitors and chemotherapy drugs that synergistically enhance antitumor immunity should also lead to further improvements in clinical outcomes. So, a better understanding of host-tumor interactions and tumor immune escape mechanisms is help to develop more effective cancer vaccines in the future [37].

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