The Development of Tumor Neoantigen Vaccine Immunotherapy

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Abstract. Activating the immune system to fight against cancers has long been a goal in immunology and oncology studies. Recent clinical-trial data proved that boosting the activity of endogenous T cells to destroy cancer cells has great potential in controlling the progression of a variety of human malignancies. In essence, neoantigen is at the core of tumor immunology. Autologous T lymphocytes could distinguish tumor cells from normal cells by recognizing neoantigens, which are tumor specific. Neoantigens are derived from genome somatic mutations of tumors, and there are different approaches to predict and identify them with increasing accuracy. Neoantigens are tumor specific, which are ideal and attractive targets for tumor immunotherapies; many neoantigen-based clinical trials are being carried out around the world. In this review, we will discuss the recent advances of tumor neoantigen vaccine immunotherapy, and present the potential obstacle and future direction of this approach.

1 Introduction

In recent centuries, human beings have developed many strategies to treat cancers, such as chemotherapeutic drugs and targeted therapies, which greatly improved the life quality and survival rate of cancer patient. But clinical outcomes are still poor, new therapies must be developed.

The genetic mutations in cancer genome not only provide driving force during tumorigenesis, but also provide immune system with ideal targets to recognize and eradicate cancer cells. In decades, researchers have developed many immunotherapies to enhance the anti-tumor immunity. In the first attempt in 1891, William Coley, a New York surgeon, treated cancer patients with live or inactivated *Streptococcus pyogenes* and *Serratia marcescens* by intratumoral injection. Later, it was proved that these “Coley’s toxins” can activate the immune system to cause inflammation and stimulate antibacterial phagocytes that might kill tumor cells through the bystander effect [1]. Now, the anti-tumor immunotherapies includes cytokines, blocking antibodies, cell therapy (TIL therapy, CAR-T cells therapy) and tumor vaccine therapy [2, 3]. In 2014, FDA approved two anti-PD1 antibodies, Pembrolizumab (Merck) and Nivolumab (Bristol-Myers Squibb), to treat patients with melanomas, which represent the beginning of a new era for cancer immunotherapy. Now, anti-PD1 antibodies were approved to treat squamous lung cancer, Hodgkin's lymphoma, bladder carcinoma, et.al. In 2017, an anti-CD19 CAR T-cell therapy, CTL019 (Novartis), was approved by FDA to treat relapsed or refractory B-cell acute lymphoblastic leukemia (ALL) in children and young adults.

Therapeutic tumor vaccines are considered to be an approach that actively stimulate the anti-tumor immunity, which will increase the breadth and diversity of tumor antigen-specific T cells [4]. Many types of tumor vaccine, including tumor cell vaccine, or vaccines derived from tumor associated antigens (TAAs, such as oncofetal antigens, oncoproteins and differentiation-associated proteins) and tumorigenic virus proteins, have been tested in clinical or animal models [5, 6]. In this review, we will discuss tumor neoantigen vaccine therapies, summarize the recent advances to develop efficient neoantigen vaccines to fight against cancers.

2 Tumor somatic mutation and neoantigen

According to the classical oncology theory, tumorigenesis is driven by multiple somatic mutations [7]. With the help of high-throughput sequencing technology, we can now get a comprehensive landscape of somatic mutations in individual tumors (the "mutanome") [8]. In particular, the novel whole exome sequencing (WES) can identify genetic variants in exomes that will alter protein sequences at a much lower cost than whole-genome sequencing. In general, melanoma, non-small-cell lung cancer (NSCLC), colorectal cancer and bladder cancer have high mutation rate, compared with other cancers [9]. Mutations result in changes in protein sequences are called missense or nonsynonymous mutations. It was found that there were dozens to hundreds of nonsynonymous mutations in different cancers, and even thousands in melanoma and lung cancers [10]. Mutations that happened in oncoproteins are called “driver mutations”, such as mutations in PTEN, EGFR and p53, which will promote

the proliferation or resistance to apoptosis of tumor cells. "Driver mutations" are highly relevant to the initiation or progression of the tumors. In fact, the majority of nonsynonymous mutations are "passenger mutations", which have no influences on tumorigenesis [11].

Some epitopes with mutation that derived from mutant protein can be presented on the surface of tumor cells by forming MHC-peptide complex, these mutant epitopes are called neoantigens [12]. These neoantigens can be recognized by T cells as "non-self" or foreign, which will help to avoid the risk of autoimmunity [13]. Researchers found that tumors with more neoantigen burden had more tumor infiltrating lymphocytes (TILs) and were more likely to benefit from anti-PD1 therapy than those with a lower mutation load [14, 15].

3 Neoantigen prediction and identification

The attempt to identify and characterize these antigenic epitopes recognized by T cells on human cancers started from the cloning of MAGEA1 in 1991, the first gene reported to induce a CTL activity [16]. Over the past few decades, a large variety of tumor specific neoantigens have been identified.

Identification of neoantigen is the key step to the development of neoantigen vaccine therapies. Previously, researchers applied an inefficient, laborious and time-consuming approach to identify neoantigens [17]. In this method, pooled cDNA library derived from cancer cells is transfected into cell line that stably express corresponding MHC molecules, and then co-cultured with T cells. The epitopes result in T cell activation, mainly measured mainly through cytokine release or 4-1BB expression, can be identified. Now, with the fast development of next generation sequencing (NGS) and mass spectrometry technology, neoantigens can be easily and precisely predicted or directly identified.

3.1 Next generation sequencing based method

WES is an efficient and economical way to identify mutations in protein coding regions. Combined with RNA sequencing (RNA-Seq) and machine learning-based prediction algorithms, researchers can predict neoantigens with high accuracy [18]. Prediction of the binding affinity of candidate peptides to MHC molecules is a critical step. Several online prediction websites have been developed, such as IEDB, NetMHCpan and NetMHCcons. Because the intracellular epitope processing is very complex, human HLA molecule is also very polymorphic, this approach is now restricted by the accuracy of MHC binding prediction algorithms [19, 20]. So, administration of multiple neoantigen vaccines can offset the inaccuracy of the prediction algorithms. In 2015, Carreno and colleagues used a tandem minigene encoding about 7 neoantigens in one expression vector as vaccine to boost the anti-tumor immunity in patient with melanoma [21].

3.2 Mass spectrometry based method

The MHC-peptide complex can be immunoprecipitated from soluble tumor lysates by the anti-HLA monoclonal antibody, and peptides can be eluted and purified for sequencing by liquid chromatography and tandem mass spectrometry (LC–MS/MS), peptide sequences can be searched against germline protein library to identify the mutations. Compared with NGS based approach, LC–MS/MS is a direct method to comprehensively analyze the repertoire of tumor neoantigens [22, 23]. Neoantigens detected by this method is real target of T cells, which will induce potent anti-tumor immunity. To predict neoantigens with more accuracy, Yadav and colleagues developed a strategy that combines WES, RNA-seq data with mass spectrometry to identify neoantigens [24].

4 Advances of neoantigen vaccine therapy

In 1995, Mandelboim and colleagues showed that immunization with synthetic peptides from mutated gap-junction protein Connexin 37 could induce antitumor CTLs and protect mice from spontaneous tumor metastasis and reduce metastatic load in the malignant 3LL-D122 murine lung carcinoma model [20]. Results also demonstrated that anti-tumor immunity was primarily mediated by CD8+ T cells. This is the first report that peptides vaccine therapy may be a promising modality for cancer therapy. In 2012, Castle and colleagues used high-throughput sequencing technology to identify somatic mutations in the mouse melanoma tumor cell line B16F10 and applied an algorithm to predict potential immunogenic epitopes [18]. In the next, they subcutaneously injected synthetic neoantigen peptides in the B16F10 melanoma tumor model. Results showed that this neoantigen vaccine can suppress the tumor progression both prophylactically and therapeutically.

In humans, there are many clinical trials attempt to test the effect of tumor neoantigen therapies. Recently, researchers tested therapeutic neoantigen vaccine consisted of peptides derived from human papillomavirus (HPV), a known causative agent of vulvar intraepithelial neoplasia. About 50% of the 19 patients who have received up to 4 vaccinations developed and maintained a complete response lasting for 2 years or longer [25]. Sampson and colleagues immunized glioblastoma patients with peptide vaccine containing an epidermal growth factor receptor variant III (EGFRvIII)-specific mutant epitope. These patients was diagnosed glioblastoma carried a common in-frame deletion of the EGFRvIII protein. Results showed that this neoantigen vaccine is safe and capable of potentially eliminating EGFRvIII-expressing tumor cells in the majority of patients. Of note, the authors also found that more than 90% of recurrent tumors showed dramatically reduced or absent expression of EGFRvIII by immunohistochemistry staining [26]. So, it indicated that immunization of a single mutated tumor antigen can lead

to immune escape.

Previously, the majority of researches mainly used MHC class I restricted neoantigen peptide as vaccines. In 2015, Kreiter and colleagues proved that the MHC class II restricted epitopes which recognized by CD4+T cells also played important role in the anti-tumor immunity [27]. Vaccination with such MHC class II restricted neoantigens induced strong antitumor activity in three independent murine tumour models.

In 2017, Catherine J. Wu and colleagues combined WES, RNA-seq and machine learning based algorithms to predict candidate neoantigens from melanoma patients, then they synthesized peptides of 15-29aa in length containing the mutant amino acids and administered them with poly-ICLC adjuvant to patients by subcutaneous injection [28]. Results showed that these vaccines induced polyfunctional CD4+ and CD8+ T cells targeted the neoantigens used in patients. Of 6 treated patients, 4 had no recurrence after vaccination in 2 years. Two patients with recurrent diseases were then treated with anti-PD1 checkpoint inhibitor antibody (pembrolizumab) and experienced complete tumor regression later.

In another clinical study that leaded by Ugur Sahin, they used nearly the same strategy to predict neoantigens from melanoma patients [29]. Based on extensive studies [30, 31], they used mRNA coding the personalized neoantigens as vaccine and directly injected into patients' inguinal lymph nodes with the assistance of ultrasonic guidence. Results showed that all 13 patients developed potent T cell responses against multiple vaccine neoantigens, 8 of them had no radiologically detectable lesions up to 23 months, and another 2 patient also experienced complete response after additional anti-PD1 therapy. In sum, these mRNA-based neoantigen vaccine induced immune responses against melanoma over 2 years and also increased the diversity of repertoire of neoantigen-specific T cells.

5 Conclusion remarks

Now, we mainly applied two strategies in the tumor immunotherapy, one is to "release the brake", such as checkpoint inhibitor therapy or adoptive T cell transfer; the other is to actively boost the antitumor activity, such as therapeutic vaccines [32, 33]. Personalized neoantigen vaccines can prime and activate DCs to stimulate robust anti-tumor T cell immunity. Several questions need to be solved to achieve the best therapeutic effects. The first one is how to improve the neoantigen prediction accuracy. So far, the most commonly used approach for identifying T cell neoepitopes is to predict peptides binding affinity to the cleft of patient-specific class I HLA molecules. Though LC–MS/MS based method has many advantages in comprehensively analyzing the repertoire of tumor neoantigens, its sensitivity need to be further improved. Second, as a personalized therapy, tumor neoantigens will spend weeks on the mutation identification, neoantigen prediction and manufacture. So, whether the neoantigen vaccine can be produced in a timely and cost-effective manner is critical in the clinical use. Third, neoantigen vaccine is very personalized and need multiple-step procession, so standardization is necessary. What is more, based on the clinical data available till now, by maximizing the antitumor functions, neoantigen vaccine plus checkpoint inhibitor antibody combination therapy strategy is very promising. In sum, with rapid advances of many relevant fields, neoantigen vaccine therapy is poised to generate a potent antitumor immunity.

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